Form Approved Through 6/30/2012 OMB No.—0925-00(

Department of Health and Human Services
Health Service

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PI: **KREIPKE, CHRISTIAN W** Council: 05/2011
1 U01 NS072045-01 A1
Dual:
IRG: ZNS1 SRC(99) Received: 11/03/2010

1. TITLE OF PROJECT (Do not exceed 81 characters, including spaces and punctuation.)
Clazosentan: A Novel Treatment of Traumatic Brain Injury

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☐ NO ☒ YES
(If "Yes," state number and title)
Number: PAR-08-233 Title: NINDS Cooperative Program in Translational Research Single-Component Reseaerch Projects (U01)

3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR

3a. NAME *(Last, first, middle)*	3b. DEGREE(S)	3h. eRA Commons User Name
Kreipke, Christian W*	PhD	aa5930

3c. POSITION TITLE: Assistant Professor

3d. MAILING ADDRESS *(Street, city, state, zip code)*:
Department of Anatomy & Cell Biology
Wayne State University
School of Medicine
540 E. Canfield, Room 9312
Detroit, MI 48201
E-MAIL ADDRESS: ckreipke@med.wayne.edu

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT: Anatomy & Cell Biology

3f. MAJOR SUBDIVISION: Medicine

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: (313)577-1049 FAX: (313)577-3125

4. HUMAN SUBJECTS RESEARCH ☒ No ☐ Yes
4a. Research Exempt ☐ No ☐ Yes If "Yes," Exemption No.
4b. Federal-Wide Assurance No.: a00002460
4c. Clinical Trial ☒ No ☐ Yes
4d. NIH-defined Phase III Clinical Trial ☐ No ☐ Yes

5. VERTEBRATE ANIMALS ☐ No ☑ Yes
5a. Animal Welfare Assurance No. A3310--01

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year–MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
07/01/11	06/30/15	914,913	1,345,699	3,871,637	4,397,008

9. APPLICANT ORGANIZATION
Name: Wayne State University
Address: Sponsored Programs Administration
5057 Woodward Ave, Suite 6402
Detroit, MI 48202

Insitutional Profile File Number (if known)

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☐ State ☐ Local
Private: → ☐ Private Nonprofit
For-profit: → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
1386028429A1
DUNS No. 00-196-2224 | Congressional District 13th

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name: Gail Ryan
Title: Sr. Director, Sponsored Programs Administration
Address: Wayne State University
Sponsored Programs Administration
5057 Woodward Ave., 13th flr., Rm. 13202
Detroit, MI 48202
Telephone (313) 577-2294 FAX (313) 577-2653
E-Mail orspsmail@wayne.edu

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: Lisa M. Ellis
Title: Grants and Contracts Officer III
Address: Wayne State University
Sponsored Programs Administration
5057 Woodward Ave., 13th flr., Rm. 13202
Detroit, MI 48202
Telephone (313) 577-9120 FAX (313) 577-505
E-Mail ak5050@wayne.edu

15. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 13. *(In ink. "Per" signature not acceptable.)* Lisa Ellis DATE 10/29/10

Use only if preparing an application with Multiple PDs/PIs. See http://grants.nih.gov/grants/multi_pi/index.htm for details.

Contact Program Director/Principal Investigator (Last, First, Middle): Kreipke, Christian W.

3. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. NIH Commons User Name
Armstead, William, M.	Ph.D.	ARMSTEADW

3c. POSITION TITLE Research Professor	3d. MAILING ADDRESS *(Street, city, state, zip code)* Dept. of Anesthesiology and Critical Care 3620 Hamilton Walk; 339 John Morgan Bldg. Philadelphia, PA 19104-6112
3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT Anesthesia	
3f. MAJOR SUBDIVISION School of Medicine	
3g. TELEPHONE AND FAX *(Area code, number and extension)* TEL: 215-573-3674 FAX: 215-349-5078	E-MAIL ADDRESS: William.armstead@uphs.upenn.edu

3. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. NIH Commons User Name

3c. POSITION TITLE	3d. MAILING ADDRESS *(Street, city, state, zip code)*
3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT	
3f. MAJOR SUBDIVISION	
3g. TELEPHONE AND FAX *(Area code, number and extension)* TEL: FAX:	E-MAIL ADDRESS:

3. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. NIH Commons User Name

3c. POSITION TITLE	3d. MAILING ADDRESS *(Street, city, state, zip code)*
3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT	
3f. MAJOR SUBDIVISION	
3g. TELEPHONE AND FAX *(Area code, number and extension)* TEL: FAX:	E-MAIL ADDRESS:

3. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. NIH Commons User Name

3c. POSITION TITLE	3d. MAILING ADDRESS *(Street, city, state, zip code)*
3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT	
3f. MAJOR SUBDIVISION	
3g. TELEPHONE AND FAX *(Area code, number and extension)* TEL: FAX:	E-MAIL ADDRESS:

Program Director/Principal Investigator (Last, First, Middle): Kreipke, Christian, William

PROJECT SUMMARY (See instructions):

Traumatic brain injury (TBI) is reportedly the leading cause of death and disability among children and young adults. Among multiple sequelae, TBI results in three major pathologies: 1) cerebral edema which leads to a critical rise in intracranial pressure, 2) diffuse axonal injury which brings about disruption of neural circuits underlying cognitive and motoric behaviors, and 3) alterations in the brain's microcirculation that cause a persistent state of hypoperfusion and improper delivery of vital metabolites to neural tissue. Over 25 clinical trials aimed at the first two pathologies have been developed, none of which have been effective in the treatment for TBI. Therefore, novel studies leading to new clinical trials are necessary. To date no one has initiated a clinical trial addressing the third pathology, *hypoperfusion following TBI*. The present proposal provides rationale for proceeding towards a clinical trial by implementing a novel therapeutic agent, Clazosentan, to improve cerebral blood flow (CBF) and, ultimately, cognitive outcome following TBI. In order to provide strong support for obtaining FDA Investigation of a New Drug (IND) status, we have designed this proposal to, first, test efficacy in a rodent model of TBI. Secondly, to more closely model the human condition, we will repeat the same efficacy measures in a porcine model of TBI. CBF will be obtained in rats and pigs using arterial spin labeling MRI (ASL), a technique used in the clinical setting, and both histopathological and cognitive outcomes will be assessed using similar paradigms across species. The data gained from this work will be used to proceed towards a Phase II-B clinical trial to effectively use Clazosentan in an effort to improve the lives of those suffering the effects of TBI.

RELEVANCE (See instructions):

Millions of dollars for hospitalization and rehabilitation and much physical and emotional deficits are incurred as a results of TBI. However, currently no effective therapies have been designed to treat the symptoms of TBI. This project will test efficacy of a novel therapy, Clazosentan, for reducing the extent of hypoperfusion to the brain which will, in turn, improve outcome. In doing so, the expected outcome of this proposal will be to receive IND from FDA to move towards human clinical trial to improve outcome after TBI.

PROJECT/PERFORMANCE SITE(S) (if additional space is needed, use Project/Performance Site Format Page)

Project/Performance Site Primary Location			
Organizational Name: Wayne State University			
DUNS: 001962224			
Street 1: 5057 Woodward	Street 2: 6th Floor		
City: Detroit	County: Wayne	State: MI	
Province:	Country: USA	Zip/Postal Code: 48202	
Project/Performance Site Congressional Districts: MI-013			
Additional Project/Performance Site Location			
Organizational Name: Trustees of the University of Pennsylvania			
DUNS: 042250712			
Street 1: 3451 Walnut Street	Street 2: P-221 Franklin Building		
City: Philadelphia	County: Philadelphia	State: PA	
Province:	Country: USA	Zip/Postal Code: 19104-6205	
Project/Performance Site Congressional Districts: PA-002			

Program Director/Principal Investigator (Last, First, Middle): Kreipke, Christian W.

SENIOR/KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Program Director(s)/Principal Investigator(s). List all other senior/key personnel in alphabetical order, last name first.

Name	eRA Commons User Name	Organization	Role on Project
Kreipke, Christian	aa5930	Wayne State Univ	Multi-PI
Armstead, William	ARMSTEADW	Univ of Pennsylvania	Multi-PI
Dore-Duffy, Paula	aa0895	Wayne State Univ	Co-Investigator
Greenberg, Joel	GREENBERG	Univ of Pennsylvania	Co-Investigator
Kuhn, Donald	aa3071	Wayne State Univ	Co-Investigator
Marguilles, Susan	MARGULIE	Univ of Pennsylvania	Co-Investigator
Mueller, Patrick	dq4607	Wayne State Univ	Co-Investigator
Rafols, Jose	JOSERAFOLS	Wayne State Univ	Co-Investigator
Smith, Douglas	SMITHD	Univ of Pennsylvania	Co-Investigator

OTHER SIGNIFICANT CONTRIBUTORS

Name	Organization	Role on Project
Goshgarian, Harry	Wayne State Univ	Conflict Resolution Negotiator
Kofke, Andrew	Univ of Pennsylvania	Conflict Resolution Negotiator
Haacke, E. Mark	Wayne State Univ	Consultant

Human Embryonic Stem Cells ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/eligibilityCriteria.asp. *Use continuation pages as needed.*

If a specific line cannot be referenced at this time, include a statement that one from the Registry will be used.

Cell Line

Program Director/Principal Investigator (Last, First, Middle): Kreipke, Christian, W.

The name of the program director/principal investigator must be provided at the top of each printed page and each continuation page.

RESEARCH GRANT

TABLE OF CONTENTS

* Follow the page limits for these sections indicated in the application instructions, unless the Funding Opportunity Announcement specifies otherwise.

Program Director/Principal Investigator *(Last, first, middle)*: **Kreipke, Christian W***

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 07/01/11 THROUGH 06/30/12

PERSONNEL *(Applicant organization only)*		Months Devoted to Project				DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mths	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Kreipke, Christian W*	Principal Investigator	3.00			157,688	39,422	10,526	49,948
Armstead, William	PD/PI-U. Pennsylvania	1.80			136,838	See Consortium Below		
Kuhn, Donald (Yr 1 only)	Co-Invest	1.80			199,700	29,955	7,998	37,953
Dore-Duffy, Paula (Yr 1 onl	Co-Invest	1.20			194,463	19,446	5,192	24,638
Rafols, Jose (Yr 1 only)	Co-Invest	1.20			187,394	18,739	5,003	23,743
Mueller, Patrick (Yr 1 only)	Co-Invest	1.20			94,728	9,473	2,529	12,002
Kropinski, Anthony (Yr 1 on	Res Asst	12.00			43,237	43,237	11,554	54,791
Graves, Justin (Yr 1 only)	Res Asst	12.00			43,237	43,237	11,554	54,791
Reynolds, Christian (Yr 1 o	Res Asst	12.00			58,065	58,065	15,503	73,568
Haacke, E. Mark (Yr 1 only	Consultant	12.00			170,021	0	0	0
*C. Kreipe (Yr 1 & 4: 3.0 cal mths; Yrs 2&3: 1.8 cal mths)								
SUBTOTALS						261,575	69,859	331,434

CONSULTANT COSTS			
Consulting Fees-Regulatory Affairs (Yr 4)		0	
Consulting Fees-Regula			0
EQUIPMENT *(Itemize)*			
Two Automated Radial Arm Mazes @ $32,000 e	0		
	0		64,000
SUPPLIES *(Itemize by category)*			
Surgical supplies (catheters, syringes, etc)	75,000		
histological reagents (FluoroJade, H&E, Fuschin)	15,000		
			90,000
TRAVEL	2 trips @ $2000/trip to Nat'l Mtg	4,000	
		0	4,000
PATIENT CARE COSTS	INPATIENT	0	0
	OUTPATIENT	0	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		0	0
OTHER EXPENSES *(Itemize by category)*			
Animals (purchase & per diem costs)	205,000		
MRI Scans (initial $71,280 & subsequent $97,720)	169,000		
Publication costs	4,000		
	0		378,000
CONSORTIUM/CONTRACTURAL COSTS - Univ Pennsylv	DIRECT COSTS	29,674	0	29,674
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD	Univ of Pennsylvania			**$ 897,108**
CONSORTIUM/CONTRACTUAL COSTS	Facilities & Administrative Costs	17,805	0	17,805
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →				**$ 914,913**

Program Director/Principal Investigator *(Last, first, middle)*: **Kreipke, Christian W***

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		331,434	30,868	31,794	54,579	0
CONSULTANT COSTS		0	0	0	35,000	0
EQUIPMENT		64,000	0	0	0	0
SUPPLIES		90,000	0	0	0	0
TRAVEL		4,000	6,180	6,365	4,371	0
PATIENT CARE COSTS	INPATIENT	0	0	0	0	0
	OUTPATIENT	0	0	0	0	0
ALTERATIONS AND RENOVATIONS		0	0	0	0	0
OTHER EXPENSES		378,000	4,120	4,244	4,371	0
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	29,674	968,312	741,683	52,401	0
SUBTOTAL DIRECT COSTS (Sum=Item 8a, Face Page)		897,108	1,009,480	784,086	150,722	0
CONSORTIUM/ CONTRACTUAL COSTS	F&A	17,805	535,987	445,010	31,440	0
TOTAL DIRECT COSTS		914,913	1,545,467	1,229,096	182,162	0
TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD						**$ 3,871,637**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Univ of Pennsylvania Program Director/Principal Investigator *(Last, first, middle)*: **Kreipke, Christian W***

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM: 07/01/11 THROUGH: 06/30/12

PERSONNEL *(Applicant organization only)*		Months Devoted to Project				DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Armstead, William*	Principal Investigator	1.80	12	0	136,838	21,141	6,533	27,674
Greenberg, Joel (YR 2-3)	Co-invest	0.00	12		121,354	0	0	0
Margulies, Susan (YR 2-3	Co-invest	0.00	12		199,700	0	0	0
Smith, Douglas	Co-invest	0.00	12		199,700	0	0	0
TBN (YR 2-3)	Technician	0.00	12		31,998	0	0	0
TBN (YR 2-3)	Technician	0.00	12		31,998	0	0	0
TBN (YR 2-3)	Technician	0.00	12		31,840	0	0	0
TBN (YR 2-3)	Technician	0.00	12		47,375	0	0	0
TBN (YR 2-3)	Technician	0.00	12		47,375	0	0	0
2 TBN (YR 2-3)	Undergrad Student @ 50% effort each	0.00	12		24,651			
*Yr 1-1.8 Cal Mths; Yrs2-4-3.0 Cal Mths)								
	SUBTOTALS →					21,141	6,533	27,674
CONSULTANT COSTS							0	0
EQUIPMENT *(Itemize)*								
Lateral Fluid Percussion Brain Injury Device (Yr 2)		0					0	
Anesthesia Machine (Yr 2)		0					0	0
Small Animal Ventilator (Yr 2)		0					0	
SUPPLIES *(Itemize by category)*								
Camera Disks & Analysis (Yr 2 & 3 only)		0					0	
Research Supplies (Yr 2 & 3 only)		0					0	
Miscellaneous lab supplies (Yr 2 & 3 only)		0		0			0	
Histopathology Supplies (Yrs 2 & 3 only)		0		0			0	
		0		0			0	
		0					0	0
TRAVEL	Travel funds for PI to/from WSU & attendance @ Sci Mtg						2,000	
							0	2,000
PATIENT CARE COSTS	INPATIENT						0	0
	OUTPATIENT						0	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*							0	0
OTHER EXPENSES *(Itemize by category)*								
Animal costs-purchase & per diem (Yrs 2-3)		0		0			0	
Surgery costs (Yrs 2-3) w/surgical suite rental		0		0			0	
Scanning costs (80 pigs Yr 2 & 40 pigs Yr 3)		0					0	
Behavioral study costs (Yrs 2-3)		0					0	0
Camera disk & miscellaneous supplies (Yr 3)		0						
CONSORTIUM/CONTRACTURAL COSTS	DIRECT COSTS						0	0
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD								**$ 29,674**
CONSORTIUM/CONTRACTUAL COSTS	Facilities & Administrative Costs						0	0
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*								**$ 29,674**

Univ of Pennsylvania — Program Director/Principal Investigator *(Last, first, middle)*: **Kreipke, Christian W***

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		27,674	381,312	391,183	50,401	0
CONSULTANT COSTS		0	0	0	0	0
EQUIPMENT		0	75,000	0	0	0
SUPPLIES		0	180,000	181,500	0	0
TRAVEL		2,000	6,000	6,000	2,000	0
PATIENT CARE COSTS	INPATIENT	0	0	0	0	0
	OUTPATIENT	0	0	0	0	0
ALTERATIONS AND RENOVATIONS		0	0	0	0	0
OTHER EXPENSES		0	326,000	163,000	0	0
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	0	0	0	0	0
SUBTOTAL DIRECT COSTS (Sum=Item 8a, Face Page)		29,674	968,312	741,683	52,401	0
CONSORTIUM/ CONTRACTUAL COSTS	F&A	17,805	535,987	445,010	31,440	0
TOTAL DIRECT COSTS		47,479	1,504,299	1,186,692	83,841	0

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$ 2,822,310**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Budget Justification

Wayne State University (Year 1)

1. Christian Kreipke, Ph.D. (3.0 CM Year 1; 1.8 CM Years 2-3; 3.0 CM Year 4), an expert in both hemodynamic and behavioral changes in rats following TBI, will have overall responsibility for AIM 1, which deals with rat experimentation at Wayne State University contained within this proposal. This includes long-term planning of goals and objectives and short-term planning of specific research protocols. While, having only 7 years experience in TBI research, Dr. Kreipke is a relatively junior member of the scientific community, he has been successful in both securing funding and has published extensively in this field. Furthermore Dr. Kreipke has developed a vast network of senior investigators to assist in this project. It should also be noted that Dr. Kreipke took the initiative to negotiate with Actelion an MTA and has subsequently negotiated all terms of drug discovery with their research department. He will perform many of the experiments and review research data on a regular, usually daily, basis. He will take the lead in the coordination of experiments conducted with rats at Wayne State with those done at the University of Pennsylvania using pigs. He will also take the lead in writing all manuscripts resulting from the use of rats in this research project. Dr. Kreipke will also serve as the contact PI for this proposal.

2. William M. Armstead, Ph.D. Multi Principal Investigator (1.8 CM Year 1; 3.0 CM Years 2-4), an expert in hemodynamic changes in pigs following TBI, will have overall responsibility for the Aims dealing with pig experimentation at the University of Pennsylvania contained within this proposal. This includes long-term planning of goals and objectives and short-term planning of specific research protocols. He will perform many of the experiments and review research data on a regular, usually daily, basis. He will take the lead in the coordination of experiments conducted with rats at Wayne State with those done at the University of Pennsylvania using pigs. He will also take the lead in writing all manuscripts resulting from the use of pigs in this research project.

Co-Investigators (*Year 1 rat studies*)

3. Donald Kuhn, Ph.D. (1.8 CM Year 1) is an expert in pharmacology and behavioral pharmacology. He will assist in designing experiments related to drug injection paradigms and will offer his expertise should issues arise from injection delivery to dosing regime.

4. Paula Dore-Duffy, Ph.D., (1.2 CM Year 1) is an expert in assessing hemodynamic changes in the brain after insult. She will assist in collecting the ASL-MRI data and will offer her expertise in interpreting the results.

5. Ewart Mark Haacke, Ph.D., (consultant, 0% effort) is a world leading authority in MRI analysis. He will assist both Drs. Kreipke and Dore-Duffy should any issues arise in the interpretation of ASL data.

6. Jose Rafols, Ph.D. (1.2 CM Year 1) is an expert in assessing histopathological outcomes in models of stroke and TBI. He will personally conduct much of the histological analysis that has been added to this revision, which includes both qualitative and quantitative analyses.

7. Patrick Mueller, Ph.D. (0.6 CM Year 1) is an expert in cardiophysiology and will assist in injection dosing and paradigm should any peripheral cardiovascular disruption ensue.

8. Anthony Kropinski, B.S. (12 CM Year 1) is a research assistant in our laboratory and has extensive experience in collecting ASL-MRI data and will be instrumental in carrying out all data collection related to CBF.

9. Justin Graves, B.S. (12 CM Year 1) is a research assistant in our laboratory and has extensive experience in both performing TBI surgery in rat and in collecting behavioral data.

10. Christian Reynolds, B.S. (12 CM Year 1) is a research assistant in our laboratory and has extensive experience in both performing histological techniques as outlined in the proposal and in collecting behavioral data. Due to the time-intensive nature of behavioral data acquisition, he will assist Mr. Graves in carrying out nightly behavioral runs.

11. Equipment. While Dr. Kreipke's laboratory is currently equipped with an automated radial arm maze, 2 more radial arm mazes will be purchased to enhance the ability to carry out large numbers of behavioral measurements as mandated by the scientific plan.

12. Travel. Funds for travel to University of Pennsylvania for direct interaction and travel to one scientific meeting each year is requested for the Multi-PI.

13. Other Direct Costs

13a. Animals. Costs are included for the purchase of rats and per diem for survival surgery brain injury studies using ASL MRI and behavior as indices of outcome.

13b. Surgery. Rental of the sterile surgical suite and a modest request for maintaining surgical supplies (sutures, scalpels, gauze, cotton, replacement hair clipper blades, etc.) is included in the budget.

13c. Scanning Costs. Initially all animals need to be scanned (396 X 1 hours X 180 per hour) = 71,280. Subsequently, a subset of 6 animals per group will be selected and scanned 3 times for a total of 288 hours worth of scans at 180 per hour = 51,480.

13d. Histology Costs. To improve the current proposal we have, in accordance with study section's suggestions, added histological analyses in all experiments. Therefore we are requesting 15,000 in year 1 to cover expenses incurred for stains, slides, other reagents, etc.

University of Pennsylvania (Year 2 and 3)

1. William M. Armstead, Ph.D. Multi Principal Investigator (1.8 CM Year 1; 3.0 CM Years 2-4), an expert in hemodynamic changes in pigs following traumatic brain injury, will have overall responsibility for the Aims dealing with pig experimentation at the University of Pennsylvania contained within this proposal. This includes long-term planning of goals and objectives and short-term planning of specific research protocols. He will perform many of the experiments and review research data on a regular, usually daily, basis. He will take the lead in the coordination of experiments conducted with rats at Wayne State with those done at the University of Pennsylvania using pigs. He will also take the lead in writing all manuscripts resulting from the use of pigs in this research project.

2. Christian Kreipke, Ph.D. (3.0 CM Year 1; 1.8 CM Years 2-3; 3.0 CM Year 4), an expert in both hemodynamic and behavioral changes in rats following TBI, will have overall responsibility for AIM 1, which deals with rat experimentation at Wayne State University contained within this proposal. This includes long-term planning of goals and objectives and short-term planning of specific research protocols. While, having only 7 years experience in TBI research, Dr. Kreipke is a relatively junior member of the scientific community, he has been successful in both securing funding and has published extensively in this field. Furthermore Dr. Kreipke has developed a vast network of senior investigators to assist in this project. It should also be noted that Dr. Kreipke took the initiative to negotiate with Actelion an MTA and has subsequently negotiated all terms of drug discovery with their research department. He will perform many of the experiments and review research data on a regular, usually daily, basis. He will take the lead in the coordination of experiments conducted with rats at Wayne State with those done at the University of Pennsylvania using pigs. He will also take the lead in writing all manuscripts resulting from the use of rats in this research project. Dr. Kreipke will also serve as the contact PI for this proposal.

3. Joel Greenberg, Ph.D. Co-Investigator (1.2 CM) will provide overall supervision for studies directed at the ASL MRI determination of cerebral blood flow in pigs after fluid percussion brain injury.
Dr. Greenberg has had extensive experience in quantitative imaging in studies of ischemia and will ensure the quality of the data obtained. He will work closely with the technician who will transport the animal to the scanner, prepare the pig for scanning, and acquire the scanning data. The data obtained will be analyzed by Dr. Greenberg who will interact with Dr. Armstead in the interpretation of these data.

4. Susan Margulies, Ph.D. Co-Investigator (1.2 CM) will provide overall supervision of behavioral studies in the pig regarding the effects of fluid percussion brain injury. Dr. Margulies has had extensive experience in behavioral techniques for detecting changes associated with brain injury in the pig.

5. Douglas Smith, MD, Co-Investigator (1.2 CM) will provide overall supervision of histopathologic studies in the pig regarding the effects of fluid percussion brain injury. Dr. Smith is a leading authority in histological analysis of brain injury in the pig.

6. To Be Named Technician (12.0 CM) will provide technical support during surgical procedures associated with induction of fluid percussion brain injury in the pig using aseptic survival surgery technique.

7. To Be Named Technician (12.0 CM) will provide technical support during surgical procedures associated with the ASL MRI determination of cerebral blood flow in pigs after fluid percussion brain injury. This individual will anesthetize the pig, transport him to the scanner, prepare him for scanning, and will obtain all scanning data. This technician will work directly with Dr. Greenberg in analyzing the data acquired.

8. Two To Be Named Technicians (12.0 CM each) who will provide technical support in the determination of the behavioral effects of fluid percussion brain injury in the pig. These individuals will work closely with Dr. Margulies.

9. To Be Named Technician (12.0 CM) will provide technical support during histological procedures associated with the newly added histological component in pigs after fluid percussion brain injury. This individual will cut tissue and process for H & E, AF, or FJ as necessary. This technician will work directly with Dr. Smith in analyzing the data acquired.

10. Two To Be Named Undergraduate Students (no cost) who will help in the technical support in the determination of the behavioral effects of fluid percussion brain injury in the pig.

11. Equipment. A modest request for a dedicated lateral fluid percussion brain injury device is requested since it would be cumbersome and difficult to move on a continual rotating basis the current device used for acute studies between the Armstead lab and a sterile surgical suite for survival studies.

12. Travel. Funds for travel to Wayne State University for direct interaction and travel to one scientific meeting each year is requested for the Multi-PI and the Co-Investigators.

13. Other Direct Costs

13a. Animals. Costs are included for the purchase of pigs and per diem for survival surgery brain injury studies using ASL MRI and behavior as indices of outcome.

13b. Surgery. Rental of the sterile surgical suite and a modest request for maintaining surgical supplies (sutures, scalpels, gauze, cotton, replacement hair clipper blades, etc.) is included in the budget.

13c. Scanning costs: In year 2, 80 pigs will be studied, while in year 3, 40 pigs will be studied. Each pig will have three scanning sessions, first prior to TBI, then four hours after treatment with Clazosentan (six hours after TBI), and finally a repeat scan two days after TBI. Each scanning session will last for 60 minutes and will entail an arterial spin label (ASL) scan for measurement of regional cerebral blood flow and a T1-weighted image for structural information. Scans will be obtained using a 3 Tesla Siemens TIM Trio located in the Center for Advanced Magnetic Resonance and Specroscopy and managed by the Center for Functional Neuroimaging. The pig head can be scanned using an a volume transmit/8-channel receive knee coil. This scanner runs pseudo-ASL and provides whole-brain CBF with 4 mm isotropic resolution. The scan costs $350/hr which includes use of the preparation suite, and full use of the scanner suite. In year 2 the use of the scanner will be $84,000 (80 pigs X 3 scanning sessions X 1.0 hrs/scanning session X $350/hr), and in year 3 it will be $42,000 (40 pigs x 3 scanning sessions X 1.0 hrs/scanning session X $350/hr).

13d. Behavioral Study costs: In year 2, 80 pigs will be studied, while in year 3, 40 pigs will be studied. Each pig will have two behavioral sessions, the cost of each session is $50. In year 2 the cost will be $8,000 (80 pigs X 2 sessions X $50/session), and in year 3 it will be $4,000 (40 pigs x 2 sessions X $50/session). In addition, in year 2, $4,000 is needed to cover cost of camera disks and 2 dedicated analysis stations. In year 3, $500 is required for camera disks and miscellaneous supplies.

13e. Histopathologic Study costs: In years 2 and 3 $25,000 will be need to purchase supplies needed for histologic analysis.

Year 4

In addition to salary requests for Multi-PIs for two months (as outlined above), 35,000 is being included for consulting fees with Regulatory Affairs in Southfield, MI for application to IND

Principal Investigator/Program Director (Last, First, Middle): Kreipke, Christian W.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Christian W. Kreipke	POSITION TITLE Assistant Professor
eRA COMMONS USER NAME aa5930	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Wayne State University	B.A.	1995-1999	Anthropology
Wayne State University	M.A.	1999-2000	Medical Anthropology
Wayne State University, School of Medicine	Ph.D.	2000-2004	Neuroscience
Wayne State University, School of Medicine	Postdoc	2004-2007	TBI

A. Personal Statement

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults. TBI results in 3 major pathologies: 1. Cerebral edema which leads to elevated ICP, 2. Diffuse axonal injury which brings about disruption of neural circuits underlying cognitive behavior, and 3. Alterations in the brain's microcirculation that cause persistent hypoperfusion and improper delivery of vital metabolites to neural tissue. While clinical trials aimed at the first two pathologies have been developed, to date none has addressed the third pathology, hypoperfusion following TBI. The present proposal uses a novel antagonist of endothelin-1 receptor A (ETrA), Clazosentan, to improve CBF and ultimately cognition. My laboratory has been primarily interested in how different endothelin receptor antagonists impact both CBF and behavioral outcome following TBI. This work includes published proof of concept data that supports the use of ETrA antagonists as a means to decrease the extent of hypoperfusion following TBI. Therefore, this proposal is a logical extension of previous work using BQ-123, a non clinically relevant ETrA antagonist, that has translational potential.

B. Positions and Honors

Positions and employment

01/97-05/97 Wayne State University, School of Medicine and Hutzel Hospital, Research Assistant, Bone Densitometry/Osteoporosis Project

09/97-09/99 Wayne State University, Institute for Information and Technology, Research Assistant, HIV/AIDS in Detroit Project

09/99-05/00 Wayne State University, Graduate Teaching Assistant, Department of Anthropology

05/00-09/00 Wayne State University, Adjunct Instructor, Department of Anthropology

09/00-08/04 Wayne State University, School of Medicine, Pre-Doctoral Research Assistant, National Institute of Drug Abuse T32 Training Grant

08/04-04/08 Wayne State University, School of Medicine, Research Associate, Dept. Anatomy and Cell Biology, Traumatic Brain Injury

04/08-present Wayne State University, School of Medicine, Research Scientist, Dept. Anatomy and Cell Biology

Other Experience and Professional Memberships

05/99-present	Member, Phi Beta Kappa
02/00-present	Member, Society for Applied Anthropology
02/00-present	Member, Society for Medical Anthropology
05/01-present	Member, Sigma Xi
05/01-present	Member, New York Academy of Sciences
05/02-present	Member, Society for Neuroscience
02/05-08/07	Wayne State Alumni Communications Committee, Committee Member
05/06-08/07	Sigma Xi, National, Associate Director, NorthCentral Region
03/07-present	Member, International Society for Cerebral Blood Flow and Metabolism
02/08-present	Member of The Royal Society of Chemistry

Honors

2002 Dean Thomas Asselin, M.D. Endowed Prize for Excellence in Psychiatry and Behavioral Neuroscience Research (Wayne State University School of Medicine)

2003 1st Place, Society for Neuroscience, MI Chapter, Poster Award

2006 Service Award For 2006 Sigma Xi National Conference

2007 Travel Award, Brain '07, Society for Cerebral Blood Flow and Metabolism

2007 Young Investigators Award, Endothelin 10, Endothelin

2010 Travel Award, Winter Brain

C. Peer-reviewed publications (from 32 selected works)

Most Relevant:

1. Shen Y, Kou Z, Kreipke CW, Petrov T, Hu J, Haacke EM. 2007. In vivo measurement of tissue damage, oxygen saturation changes and blood flow changes after experimental traumatic brain injury in rats using susceptibility-weighted imaging. Magn Reson Imaging 25:219-227.
2. Kallukuri S, Kreipke CW, Rossi N., Rafols JA, Petrov T. 2007. Spatial alterations in endothelin receptor expression are temporally associated with the altered microcirculation after brain trauma. Neurological Research 29:362-368.
3. Kreipke CW, Rafols JA. 2009. Calponin control of cerebrovascular reactivity: Therapeutic implications in brain trauma. J Cell Mol Med 13(2):262-9.
4. Kreipke CW, Schafer PC, Rossi NF, Rafols JA. 2010. Differential affects of Endothelin Receptor-A and B antagonism on hypoperfusion following traumatic brain injury (TBI). Neurological Research 32:209-214.
5. Reynolds CA, Rafols JA, Schafer S, Pirooz R, Marinica A , Chbib A, Bedford C, Fronczak M, Kuhn DM, Kreipke CW. (in press) Differential effects of endothelin receptor A and B antagonism on behavioral outcome following traumatic brain injury. Neurological Research.

Other Relevant Articles:

6. Kuhn DM, Sadidi M, Lu X, Kreipke C, Geddes T, Borges C, and Throck J. 2002. Peroxynitrite-Induced Nitration of Tyrosine Hydroxylase: Identification of Tyrosines 423, 428, and 432 as Sites of Modification

by MALDI-TOF Mass Spectrometry and Tyrosine-Scanning Mutagenesis. Journal of Biological Chemistry 277:14336-14342.

7. Kreipke CW, Morgan R, Kallikuri S, Rafols J. 2007. Behavioral preconditioning enhances angiogenesis and cognitive outcome following traumatic brain injury. Neurological Research 29:388-394.
8. Kreipke CW, Morgan R, Petrov T, Rafols JA. 2007. Subcellular Redistribution of Calponin Underlies Sustained Vascular Contractility Following Traumatic Brain Injury. Neurological Research 29:604-609.
9. Rafols J., Kreipke CW, Petrov T. 2007. Alterations in Cerebral Cortex Microvessels and the Microcirculation in a Rat Model of Traumatic Brain Injury: a Correlative EM and Laser Doppler Flowmetry Study. Neurological Research 29:339-347.
10. Rafols J, Morgan R, Kallikuri S, Kreipke CW. 2007. Extent of nerve cell injury Marmarou's model compared to other brain trauma models. Neurological Research 29:348-355.
11. Dore-Duffy P, Xeuqain W, Mehedi A, Kreipke CW, Rafols JA. 2007. Differential expression of capillary VEGF isoforms following traumatic brain injury. Neurological Research 29:395-403.
12. Huttemann M, Lee I, Kreipke CW, Petrov T. 2008. Suppression of iNOS prior to traumatic brain injury improves cytochrome oxidase activity and normalizes cellular energy levels. Neuroscience 151:148-151.
13. Kallakuri S, Kreipke CW, Schafer PC, Schafer SM, Rafols JA. 2010 Brain cellular localization of endothelin receptor A and B in a rodent model of diffuse brain injury. Neuroscience 168:820-30.
14. Armstead W, Kreipke CW. (in press) Endothelin-1 is upregulated after traumatic brain injury: A cross-species, cross-model analysis. Neurological Research
15. Dore-Duffy P, Ding Y, Zhan P, Schafer S, Fronczak M, Rafols JA, Kreipke CW. (in press) Endothelin receptor expression in pericytes following ETrA antagonist treatment. Neurological Research.

D. Research Support

Ongoing Research Support

R01 NS064976-A2 Kreipke (PI) 11/01/09-10/31/14
NIH_NINDS
Role: PI
"Molecular Mechanisms of Enhanced Contractility following Traumatic Brain Injury: towards a clinical trial" (Investigates the mechanism by which endothelin receptor antagonists may be useful in the treatment of cognitive deficits following TBI).

VARR&D 1I01RX000224-01 Kreipke (PI) 11/01/09-10/31/12
Role: PI
"Poly-trauma following brain injury: towards a combinatorial therapy" (Investigates the effects of multiple pathologies associated with traumatic brain injury on histopathological and behavioral outcome).

VA RR & D Award Rossi/Kreipke (PI) 04/01/08-12/31/11
VA Rehabilitation
Role: CO-PI
"Conditioning, microvascular tone & rehabilitation post brain trauma" (Investigates the role of exercise in the control of microcirculation in a rat model of traumatic brain injury).

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
William M. Armstead, Ph.D.	Research Professor
eRA COMMONS USER NAME ARMSTEADW	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Pennsylvania, Philadelphia, PA	B.A.	05/79	Biochemistry
Tulane University, New Orleans, LA	M.S.	05/83	Pharmacology
Tulane University, New Orleans, LA	Ph.D.	05/85	Pharmacology
Tulane University, New Orleans, LA	Post-Doc	05/86	Pharmacology
University of Tennessee, Memphis, TN	Post-Doc	05/88	Physiology

A. Personal Statement

Traumatic brain injury (TBI) is the leading cause of death and disability among young adults. TBI results in 3 major pathologies: 1. Cerebral edema which leads to elevated ICP, 2. Diffuse axonal injury which brings about disruption of neural circuits underlying cognitive behavior, and 3. Alterations in the brain's microcirculation that cause persistent hypoperfusion and improper delivery of vital metabolites to neural tissue. While clinical trials aimed at the first two pathologies have been developed, to date none has addressed the third pathology, hypoperfusion following TBI. The present proposal uses a novel antagonist of Endothelin-1, Clazosentan, to improve CBF and ultimately cognition. My lab has been one of the few that has characterized the relationship between cerebral hemodynamics and histopathologic outcome after TBI using a pig model, thought to be more similar to that of the human. One important mediator of damage investigated by us has been endothelin-1. Therefore, this proposal is a natural progession of my research interests towards developing a novel therapeutic for treatment of TBI in the pediatric and young adult population.

B. Positions and Honors

Positions and Employment

1988-1990 Instructor, Department of Physiology and Biophysics, University of Tennessee, Memphis, TN
1990-1992 Assistant Professor, Dept of Physiology and Biophysics, U of Tennessee, Memphis, TN
1992-1999 Assistant Professor, Depts of Anesthesia and Pharmacology, U of Pennsylvania; Department of Anesthesiology & Critical Care Medicine, The Children's Hospital of Philadelphia
1999-2009 Research Associate Professor, Depts of Anesthesia and Pharmacology, U of Pennsylvania
2009-present Research Professor, Depts of Anesthesiology and Critical Care, Pharmacology, U of Penn

Other Experience and Professional Memberships

1992- Member, American Physiological Society
1992- Member, American Society of Pharmacology and Experimental Therapeutics
1992- Member, Neurotrauma Society
1992- Member, Int. Society of Cerebral Blood Flow and Metabolism
1998-2010 Editorial Board, Microcirculation
2001-2005 Chartered Member, AHA National Brain 2 Study Section
2002- Executive Board, ASPET Cardiovascular Division
2002- Member, ASPET Cardiovascular Division Student/Post Doc Best Abstract Award Committee
2005-2006 Ad Hoc Member, NIH DBD Study Section
2006- Editorial Board, American Journal of Physiology: Heart and Circulatory Physiology
2006-2007 Member, Study Section for Catalan Agency for Health Technology Assessment, Spain
2007- Chartered Member, Veterans Administration Neurobiology C Study Section
2008-2009 Ad Hoc Member, NIH ANIE Study Section

2008-2009	Member, AHA Region II Brain Study Section
2009-	Member, AHA Region I Brain Study Section
2009-	Awards Committee, Microcirculatory Society
2010-	Chair, Experimental Methods Section, AHA/ASA Int. Stroke Conference Planning Committee

Honors

1979	Phi Lambda Upsilon
1987	Sigma Xi
1994	Established Investigator Award of AHA
2003	Fellow, Cardiovascular Section of the American Physiological Society
2003	Fellow, Stroke Council of the AHA

C. Publications: (Selected from 174 manuscripts and 15 Reviews and Chapters)

Most relevant to the current application

1. Armstead WM, Kreipke CW. Endothelin-1 is upregulated after traumatic brain injury: A cross-species, cross-model analysis. Neurological Research, in press
2. Kreipke CW, Rafols JA, Reynolds CA, Schafer S, Marinica A, Beford C, Fronczak M, Kuhn D, Armstead Armstead WM. Clazosentan: An initial report on the efficacy of a novel endothelin receptor-A antagonist following traumatic brain injury. Neurological Research, in press.
3. Armstead WM. Role of endothelin-1 in pial artery vasoconstriction and altered responses to vasopressin following brain injury. J Neurosurg 85: 901-907, 1996. PMID: 8893730
4. Armstead WM. Role of endothelin-1 in age dependent cerebrovascular hypotensive responses after brain injury. Am J Physiol 277: H1884-H1894, 1999. PMID: 10564144
5. Armstead WM. Endothelin induced cyclooxygenase dependent superoxide generation contributes to K channel function impairment after brain injury. J Neurotrauma 18: 1039-1048, 2001. PMID: 11686491

Additional recent publications of importance to the field

1. Armstead WM, Kiessling JW, Kofke WA, Vavilala MS. Impaired cerebral blood flow autoregulation during post traumatic arterial hypotension after fluid percussion brain injury is prevented by phenylephrine in female but exacerbated in male piglets by ERK MAPK upregulation. Crit Care Med 38:1868-1874, 2010. PMID: 20562700
2. Armstead WM, Kiessling JW, Bdeir K, Kofke WA, Vavilala MS. Adrenomedullin prevents sex dependent impairment of autoregulation during hypotension after piglet brain injury through inhibition of ERK MAPK upregulation. J Neurotrauma 27: 391-402, 2010. PMID: 20170313
3. Armstead WM, Kiessling JW, Kofke WA, Vavilala MS. SNP improves cerebral hemodynamics during normotension but fails to prevent sex dependent impaired cerebral autoregulation during hypotension after brain injury. Brain Res, 1330: 142-150, 2010. PMID: 20298682
4. Armstead WM, Riley J, Kiessling JW, Cines DB, Higazi AAR. PAI-1 derived peptide EEIIMD prevents impairment of cerebrovasodilation by augmenting p38 MAPK upregulation after cerebral hypoxia/ischemia. AJP, 299: H76-H80, 2010. PMID: 20435843
5. Armstead WM, Riley J, Kiessling JW, Cines DB, Higazi AAR. Novel plasminogen activator inhibitor-1 derived peptide protects against impairment of cerebrovasodilation after photothrombosis through inhibition of JNK MAPK. Am J Physiol, 299: R480-R485, 2010. PMID: 20538898
6. Eucker SA, Hoffman BD, Natesh R, Ralston J, Armstead WM, Margulies SS. Development of a fluorescent microsphere technique for rapid histological determination of cerebral blood flow. Brain Res, 1326:128-134, 2010. PMID: 20193669
7. Philip S, O Chaiwat, Y Udomphorn, A Moore, JJ Zimmerman, WM Armstead, MS Vavilala. Variation in cerebral blood flow velocity with cerebral perfusion pressure > 40 mm Hg in 42 children with severe traumatic brain injury. Crit Care Med 37: 2973-2978, 2009. PMID: 19770734
8. Armstead WM, DB Cines, K Bdeir, Y Bdeir, SC Stein, AAR Higazi. uPA modulates the age dependent effect of brain injury on cerebral hemodynamics through LRP and ERK MAPK. J Cereb Blood Flow Metab, 29: 524-533, 2009 PMID: 19050721

9. Armstead WM, Ganguly K, Kiessling JW, Chen XH, Smith DH, Higazi AAR, Cines DB, Bdeir K, Zaitsev S, Muzykantov VR. RBC-coupled tPA prevents impairment of cerebral vasodilatory responses and tissue injury in pediatric cerebral hypoxia/ischemia through inhibition of ERK MAPK. J Cereb Blood Flow Metab, 29: 1463-1474, 2009. PMID: 19436314
10. Kiessling JW, DB Cines, AAR Higazi, WM Armstead. Inhibition of integrin $\alpha_v\beta_3$ prevents urokinase plasminogen activator-mediated impairment of cerebrovasodilation after cerebral hypoxia/ischemia. Am J Physiol, 296: H862-H867, 2009. PMID: 19168722

D. Research Support

Ongoing Research Support

NS 53410 (Armstead) 6/1/06-5/31/11 4.8 CM
NIH/NINDS $218,475
Plasminogen activators and cerebral ischemic injury
The major goals of this project are to: 1. Characterize the relationship between the plasminogen activators and cerebral hemodynamics after hypoxia/ischemia, 2. Investigate the role of MAPK as the mechanism by which plasminogen activators control cerebral hemodynamics post insult; Changes in the MAPK isoform expression profile result in impaired cerebral hemodynamics and neuron cell loss, and 3. Deteremine the association between impaired cerebral hemodynamics and histopathology post insult.

HD57355 (Armstead) 3/20/08-2/28/13 4.8 CM
NIH/NICHD $212,500
Plasminogen activators and NMDA after brain injury
The major goals of the project are to: 1. Characterize the relationship between plasminogen activators and NMDA receptor activation in cerebral hemodynamics following brain injury as a function of age, 2. Investigate the role of MAPK isoforms and LRP as the mechanism by which plasminogen activators and NMDA receptor activation control cerebral hemodynamics following brain injury as a function of age, and 3. Determine the association between plasminogen activators and NMDA receptor induced impairment of cerebral hemodynamics and histopathology following brain injury as a function of age.

HL 77760 (Higazi) 4/1/06-3/30/11 0.6 CM
NIH/NHLB $225,000
tPA in traumatic brain injury
The major goals of this project are to: 1) Study the role of tPA in traumatic brain injury; 2) Identify the receptors that mediates its signal transduction effect and to develop approaches to inhibit such pathways.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Paula Dore-Duffy	Professor of Neurology
eRA COMMONS USER NAME (credential, e.g., agency login) aa0895	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Simmons College, Boston, MA	B.S.	1972	Biology
Baylor College of Medicine, Houston, TX	--	1973	Virology
Louisiana State University, School of Medicine, New Orleans, LA	Ph.D	1978	Microbiology / Immunology

A. Personal Statement

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults. TBI results in 3 major pathologies: 1. Cerebral edema which leads to elevated ICP, 2. Diffuse axonal injury which brings about disruption of neural circuits underlying cognitive behavior, and 3. Alterations in the brain's microcirculation that cause persistent hypoperfusion and improper delivery of vital metabolites to neural tissue. While clinical trials aimed at the first two pathologies have been developed, to date none has addressed the third pathology, hypoperfusion following TBI. The present proposal uses a novel antagonist of Endothelin-1, Clazosentan, to improve CBF and ultimately cognition. Dr. Kreipke and I have worked closely on several projects regarding blood flow, angiogenesis, and BBB. Further, I have served on numerous study sections for the National Institutes of Health, Department of Defense, and the Veterans' Administration Hospitals in the MS, immunology, and vascular biology fields. Over the years, I have had considerable experience running a research program at the R01 level and Center grant. I was recently awarded one of the MS Society's Collaborative Research Center Awards that coordinates four projects. I therefore have the background and expertise to lead the proposed research program.

B. Positions and Honors

Positions and Employment

1979-1982 Assistant Professor, Neurology and Medicine, University of Connecticut School of Medicine, Farmington, CT
1982-1988 Associate Professor, Medicine, University of Connecticut School of Medicine, Farmington, CT
1982-1988 Associate Professor, Neurology, University of Connecticut School of Medicine, Farmington, CT
1982-1988 Director, UCHC MS Center, University of Connecticut School of Medicine, Farmington, CT
1977 Tenure, University of Connecticut School of Medicine, Farmington, CT
1984-1988 Co-Director, Neuroscience Graduate Program, University of Connecticut School of Medicine, Farmington, CT
1988-Pres Co-Director, Multiple Sclerosis Center, Wayne State University School of Medicine Detroit, MI
1989-Pres Chief, Division of Neuroimmunology, Department of Neurology, Wayne State University School of Medicine Detroit, MI
1988-Pres Professor, Neurology, Wayne State University School of Medicine Detroit, MI

1988-Pres Associate Professor, Immunology and Microbiology, Wayne State University School of Medicine, Detroit, MI
1996-1998 Co-Director, Detroit Neurotrauma Center, Wayne State University School of Medicine, Detroit, MI

Other Experience and Professional Memberships
National Institutes of Health, Study Section Reviewer, BINP, 2005-2007
American Heart Foundation, Reviewer, 2007
Department of Defense Neurobiology Committee A, Member, 2007
Program Committee, Winter Conference on Brain Research 2007
American Neurological Association
American Academy of Neurology
The American Association of Immunologists (AAI)
Society for Neuroscience
American Society of Neurochemistry
The Royal Society of Medicine
National Neurotrauma Society
International Society for Blood Flow & Metabolism
International Brain Barrier Society

Honors / Awards
2008 Academy of Scholars, Wayne State University
2007 Faculty Excellence in Research Award, Wayne State University School of Medicine
1998 Deputy Editor, Journal of Neurological Sciences
1998 NIH Study Section, NB4
1996 Gershenson Distinguished Faculty Fellow
1994-Pres Editorial Boards: Neurology, Microvascular Research, Journal of Experimental Microbiology and Immunology, Journal of Clinical Pharmacology
1988-1992 National Institute of Health Study Section (NSPA)
1985-1986 Visiting summer scientist Mount Desert Island Biological Labs, Salisbury Cove, Maine
1985 Kroc Foundation Endowment $50,000 to establish a yearly MS Symposium
1980 Multiple Sclerosis Society Bursar, 4th International Congress of Immunology in Paris
1979 Outstanding Young Women Scientist of America
1977-1978 National Multiple Sclerosis Society Fellowship Award

C. Selected Peer-reviewed Publications
Most relevant to the current application

Dore-Duffy, P., Donovan. C. and Todd, R.F. III. Monocyte Activation Associated Antigen MO3e in MS. Neurology. 42:1609-1615, 1992.

Washington, R., Burton J., Todd, R.F., III, Dragovic, L., and Dore-Duffy, P. Expression of immunologically Relevant Endothelial Cell Activation Antigens of Isolated CNS Microvessels from Patients with MS. Evidence for Focal Activation of Vascular Endothelium. Ann. Neurol. 35:89-97, 1994.

Washington, R. and Dore-Duffy, P. Role of Cytoskeletal Elements in Expression of Monocyte Urokinase Plasminogen Activator Receptor, Activation-Associated Antigen Mo3. Clin. Diag. Lab. Immunol. 1(6): 714-721, 1994.

Dore-Duffy, P., Balabanov, R., Washington, R., and Swanborg, R.H. Transforming Growth Factor Beta-1 Inhibits CNS Endothelial Cell Activation. Mol. Chem. Neuropath. 3:161-175, 1994

Dore-Duffy, P., Washington, R., and Balabanov, R. Cytokine-mediated activation of cultured CNS microvessel: A system for examining antigenic modulation of CNS EC and evidence for long-term expression of adhesion protein E-selectin. J. Cereb. Blood Flow Metab. 14:837-884, 1994.

Dore-Duffy, P., Balabanov, R. and Washington, R. Recovery from acute experimental autoimmune

Encephalomyelitis (EAE) characterized by endothelial cell unresponsiveness cytokines and pericytes activation. Biology and Physiology of the Blood-Brain Barrier. 57:347-351, 1996.

Dore-Duffy, P., Balabanov, R., Beaumont, T., Hritz, M.A. Harik, S.I. and LaManna J.C. Endothelial activation Following Prolonged Hypobaric Hypoxia. Microvas. Res. 57:75-85, 1999.

Balabanov, R., Dore-Duffy, P., The CNS microvascular pericyte response to hypoxia. Neurotrauma 19:1331, 2002.

Dore-Duffy, P, Balabanov R, Wang X, Beaumont T. Pericyte release of cyclopentenone prostaglandins in response to hypoxia. Microvascular Research 35; 215-226, 2005.

Dore-Duffy, P., Katychev, A., Wang, X, and Van Buren, E. CNS microvascular pericytes exhibit multipotential stem cell activity. J Cereb Blood Flow Metab. 26: 613-624, 2006.

Dore-Duffy, P. and LaManna, JC. Physiological Angiodynamics in the brain and Redox signaling. Antioxidants and Redox Signaling 9: 1363-1371, 2007.

Dore-Duffy, P., Wang, X., Mehedi, A., Kreipke, W., and Rafols, J. Differential expression of capillary VEGF isoforms following traumatic brain injury. Neurological Res. 29: 395-403, 2007.

Milner, R., Hung, S., Erokwu, B., Dore-Duffy, P., LaManna, J., and del Zoppo, G. Increased expression of fibronectin and the $\alpha 5\beta 1$ integrin in angiogenic cerebral blood vessels of mice subject to hypobaric hypoxia. Mol Cell Neurosci. 38: 43-52, 2008.

Dore-Duffy, P. Pericytes: Pluripotent cells of the blood brain barrier. Curr Pharm Des. 14: 1581-1593, 2008.

Dore-Duffy, P., Cleary, K. "Morphology and Properties of the Neurovascular Unit: The Pericyte", *in* The Blood-Barrier and Other Neural Barriers: Biology and Research Protocols, Ed: S. Nag, Humana Press Inc, Totowa (in press).

D. Research Support

Collaborative Multiple Sclerosis Research Center Award
Principal Investigator: Paula Dore-Duffy, Ph.D.
Agency: National Multiple Sclerosis Society
Period: April 1, 2007 to March 31, 2012

Poly-trauma following brain injury: towards a combinatorial therapy
Principal Investigator: Christian Kreipke, PhD
Agency: Veteran's Administration (VARR&D 1I01RX000224-01)
Period: November 1, 2009 to October 31, 2012
(Investigates the effects of multiple pathologies associated with traumatic brain injury on histopathological and behavioral outcome).

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Greenberg, Joel H.	Research Professor Neurology
eRA COMMONS USER NAME (credential, e.g., agency login) GREENBERG	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Manitoba, Winnipeg, MB. Canada	B.S.	1967	Engineering Physics
University of Pennsylvania, Philadelphia, PA	Ph.D.	1974	Biomedical Engineering

A. Personal Statement

The goal of the proposed research is to investigate the translational potential of Clazosentan, an endothelin-1A antagonist, in ameliorating hypotension and in improving behavioral outcome following traumatic brain injury (TBI). Specifically, we will use TBI models in rats (preliminary dosing and probing studies) and in pigs (a species for which a model more relevant to human TBI exists) to evaluate Clazosentan for treating TBI. I have been working in the field of cerebrovascular physiology and cerebral ischemia for over three decades utilizing ischemia models in rats, cats, and non-human primates. I have used autoradiographic, tomographic (PET, SPECT), and optical techniques to measure cerebral blood flow in models of ischemia, hypoxia, hypotension and functional activation. Early in my career I worked with Drs. Reivich and Kuhl at the University of Pennsylvania, and Dr. Wolf at Brookhaven National Laboratories to develop the 18-F-fluorodeoxyglucose technique for the measurement of cerebral glucose utilization and have used this technique to study changes in glucose metabolism in a variety of disease states and following functional activation. This has provided me with a strong background in tomographic measurements of blood flow and metabolism. More recently I have been working with Dr. John Detre from the Departments of Neurology and Radiology to migrate and validate the magnetic resonance imaging techniques for blood flow measurements routinely used in humans to the pig and the rat. Dr. Detre, who developed the arterial spin label (ASL) technique for blood flow measurement using MRI that will be used in the proposed studies, and I have been close collaborators for over ten years. I bring to this project extensive experience in the measurement of cerebral blood flow in models of cerebral ischemia and will be responsible for the ASL-MRI measurements following fluid percussion TBI in the pig.

B. Positions and Honors

Positions and Employment

1968-1968	Research Associate, Physiological Flow Studies Unit, Imperial College of Science and Technology, London, England.
1973-1975	Research Associate, Cerebrovascular Research Center, Department of Neurology, University of Pennsylvania, School of Medicine.
1975-1980	Research Assistant Professor, Dept. of Neurology, University of Pennsylvania, School of Medicine.
1980-1997	Research Associate Professor, Dept. of Neurology, University of Pennsylvania, School of Medicine
1997-present	Research Professor, Department of Neurology, University of Pennsylvania, School of Medicine

Other Experience and Professional Memberships

1978-present	Fellow-Stroke Council, American Heart Association
1997-present	Director, International Society of Cerebral Blood Flow and Metabolism

2001-2003	Chair Program Committee, Brain'03 (International Symposium of Cerebral Blood Flow, Metabolism and Function)
2005-2007	President-elect, International Society of Cerebral Blood Flow and Metabolism
2007-2009	President, International Society of Cerebral Blood Flow and Metabolism
2009-2011	Past-president, International Society of Cerebral Blood Flow and Metabolism

Honors

1967-1969	Ford Foundation Fellowship
1969-1973	Medical Research Council of Canada Studentship

C. Selected Peer-reviewed Publications (Selected from 175 peer-reviewed publications)

Most relevant to the current application

Greenberg, J.H., Reivich, M., Alavi, A., Hand, P., Rosenquist, A, Rintelmann W., Tusa, R., Stein, A., Christman, D., Fowler, J., MacGregor, B., and Wolf, A.: Metabolic mapping of functional activity in man with the ^{18}F-fluorodeoxyglucose technique. Science 212:678-680, 1981. (PMID: 6971492)

Komatsumoto, S., Nioka, S., Greenberg, J.H., Yoshizaki, K., Subramanian, V.H., Chance, B., Reivich, M.: Cerebral energy metabolism measured in vivo by 31P-NMR in middle cerebral artery occlusion in the cat - relationship to severity of stroke. J. Cereb. Blood Flow Metab. 7:557-562, 1987. (PMID: 3654795)

Dezsi, L., Greenberg, J.H., Hamar, J., Sladky, J., Karp, A., Reivich, M.: Acute improvement in histological outcome by MK-801 following focal cerebral ischemia and reperfusion in the cat independent of blood flow changes. J Cereb Blood Flow Metabol 12:390-399, 1992. (PMID: 1314841)

Lu, D., Joseph, P.M., Greenberg, J.H., Lin, R., Mukherji, B., Sloviter, H.A.: Use of 19F magnetic resonance imaging to measure local cerebral blood volume. MRM 29:179-187, 1993. (PMID: 8429781)

Greenberg JH, Araki N, Karp A: Correlation between ^{99m}Tc-bicisate and regional CBF measured with iodo-[^{14}C] antipyrine in a primate focal ischemia model. J Cereb Blood Flow Metabol 14(Suppl 1):536-543, 1994. (PMID: 8263069)

Takahashi, K., Pieper, A.A., Croul, S.E., Zhang, J., Snyder, S.H., Greenberg, J.H.: Post-treatment with an inhibitor of poly(ADP-ribose) polymerase attenuates cerebral damage in focal ischemia. Brain Res. 829:46-54, 1999. (PMID: 10350529)

Shimazu, T, Inoue I, Araki, N, Asano, Y, Sawada, M, Furuya, D, Nagoya, H, Greenberg, JH.: A peroxisome proliferator-activated receptor-γ agonist reduces infarct size in transient but not in permanent ischemia. Stroke 36:353-359, 2005. (PMID: 15618443)

Additional recent publications of importance to the field (in chronological order)

Tanaka, K., Dora., E., Urbanics, R., Greenberg, J.H., Toffano, G., Reivich, M.: Effect of the ganglioside GM1 on the cerebral metabolism, microcirculation, recovery kinetics of ECoG and histology during the recovery period following focal ischemia in cats. Stroke 17:1170-1178, 1986. (PMID: 3810717)

Uematsu, D., Greenberg, J.H., Hickey, W.F., Reivich, M.: Nimodipine attenuates both increase in cytosolic free calcium and histological damage following focal cerebral ischemia and reperfusion, Stroke 20:1531-1537, 1989. (PMID: 2815188)

Greenberg, J.H.: Glucose and Oxygen Metabolism in Ischemia. In: Cerebrovascular Disease: Pathophysiology, Diagnosis, and Management. M. Ginsberg and J. Bogouoslavsky, (eds.) Blackwell Scientific Publications, Cambridge, 1998, pp. 227-248.

Gomi, S., Karp, A., Greenberg, J.H.: Regional alterations in an excitatory amino-acid transporter, blood flow, and glucose metabolism after middle cerebral artery occlusion in the rat. Exp Brain Res 130:521-528, 2000. (PMID: 10717793)

Watanabe, S., Hoffman, JR, Craik, RL, Hand, PJ, Croul, SE, Reivich, M, Greenberg, JH: A new model of localized ischemia in the rat somatosensory cortex produced by cortical compression. Stroke 32:2615-23, 2001 (PMID: 11692026)

Durduran, T., Burnett, M.G., Yu, G., Zhou, C., Furuya, D., Yodh, A.G., Detre, J.A., Greenberg, J.H.: Spatiotemporal quantification of cerebral blood flow during function activation in rat somatosensory cortex using laser-speckle flowmetry. J Cereb Blood Flow Metab 24:518-525, 2004 (PMID: 15129183)

Zhou, C., Shimazu, T., Durduran, T., Luckl, J., Kimberg, D.Y., Yu, G., Chen, X-H., Detre, J.A., Yodh, G., Greenberg, J.H.: Acute functional recovery of cerebral blood flow following forebrain ischemia in the rat. J Cereb Blood Flow Metabol 28:1275-1284, 2008 (PMCID: PMC2771551)

Luckl, J., Zhou, C., Durduran, T., Yodh, A.G., Greenberg, J.H. : Characterization of periinfarct flow transients with laser speckle and Doppler after middle cerebral artery occlusion in the rat. J Neurosci Res 87:1219-1229, 2009 (PMID: 19006084)

C. Research Support:

Ongoing Research Support

1-RO1 NS057400 Greenberg (PI) 03/01/08 – 02/28/11
NIH/NINDS
Functional activation during cerebral ischemia

The goal of this project is to examine the neuroprotective properties of functional stimulation during cerebral ischemia.
Role: PI

1-RO1 NS060653 (Yodh) 09/01/08 – 08/31/12
NIH
Diffuse Optics for Acute Stroke Management

The major goal of this project is to develop optical tools to monitor acute stroke patients and to demonstrate the clinical utility of these tools.
Role: Subcontract PI

ITMAT (Greenberg/Dmochowski/) 10/01/08 – 09/30/10
Institute for Translational Medicine (PENN)
Validation of Target Xenon SPECT Agents for *In Vivo* Molecular Imaging

The goal of this project is to develop protocols for attaching peptide and small-molecule targeting agents to cryptophanes so as to produce compounds that can target cancer cells or be used to monitor variety of physiological functions and thus be used in emission tomography.
Role: Co-PI

Completed Research Support

URF Greenberg (PI) 07/01/04 – 6/31/05
University of Pennsylvania Research Foundation
A Portable Device to Monitor Cerebral Blood Flow, Oxygen Saturation and Oxygen Metabolism in Patients

This grant is dedicated to building a device for the non-invasive measurement of cerebral blood flow and cerebral oxygen concentrations in head-injured patients. This device will be tested in a model of focal ischemia in the cat.
Role: PI

1-R01-HD44769-01 Hoffman (PI) 09/01/03 – 06/30/06
NIH/NICHD

Enhancing recovery of sensation after cerebral ischemia
The focus of this research project is to examine factors that optimize functional recovery and maximize the anatomical and physiological reorganization of the central nervous system following central lesions.
Role: Subcontract PI

2-RO1-NS33785 Greenberg (PI) 09/01/02 – 06/30/07
NIH/NINDS
Ischemia Induced Plasticity – Implications for Therapy

The major goal of this project is to examine metabolic and behavioral reorganization following focal cerebral ischemia of the somatosensory cortex in the rat, and develop techniques for accelerating this reorganization.
Role: PI

R01 HL077699-01 Yodh (PI) 09/01/04 – 08/31/08
NIH/NHLBI
Diffuse Light Imaging of Flow, Oxygen & Brain Metabolism

The goal of this project is to design and build a versatile multi-modality all-optical imaging probe for measurement of total hemoglobin concentration, blood oxygenation, blood flow, the cerebral metabolic rate for oxygen and changes of these parameters in the brain.
Role: Subcontract PI

CNC Greenberg (PI) 02/01/07 – 01/31/08
Comprehensive Neuroscience Center
Optical monitoring of acute stroke patients

The goal of this project is to evaluate the ability of an optical imaging device to monitor cerebral auto-regulation in acute stroke patients so that their post-stroke care can be individualized
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
E. Mark Haacke, PhD	Professor and Directors
eRA COMMONS USER NAME ak5444	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Toronto	B.S.	1973	Mathematics & Physics
University of Toronto	M.S.	1975	Theoretical Physics
University of Toronto	Ph.D.	1978	High Energy Physics

A. Personal Statement

Prof. Haacke has been focusing on traumatic brain injury projects for the last five years. He has been instrumental in evaluating TBI using modern advanced imaging technologies such as susceptibility weighted imaging for example. His background is in the development and application of new imaging methods such as SWI and diffusion tensor imaging (DTI). He was involved in the NINDS/NIH 2009 workshop on TBI and has been involved in the preparation of an imaging report to the community that is currently under review. Finally, Prof. Haacke has been collaborating with all personnel on this project for the last five years as well.

B. Positions and Honors.

Positions and Employment

1981-1983 **Research Geophysicist**, Gulf Research and Development, Pittsburgh, PA.
1983-1985 **Senior Research Scientist**, Picker International, Highland Heights, OH.
1985-1989 **Assistant Professor of Radiology and Physics**, Head, MR Physics and Basic Science. Case Western Reserve University, Cleveland, OH.
1989-1993 **Associate Professor**, Department of Radiology with appointments in Physics and Biomedical Engineering, Case Western Reserve University, Cleveland, OH.
1993-1999 **Professor of Radiology**, Director MR Imaging Research, Mallinckrodt Institute of Radiology, Washington University, St. Louis, MO.
1999-Present **Director**, The MRI Institute for Biomedical Research, Detroit, MI.
2002-Present **Professor of Radiology**, Wayne State University, Detroit, MI.
2002-Present **Director**, Wayne State University, Magnetic Resonance Imaging Facility, Detroit, MI.
2002-Present **Professor of Biomedical Engineering**, Wayne State University, Detroit, MI.
2002-Present **Adjunct Professor**, Loma Linda University, Loma Linda, CA.
2005-Present **Adjunct Professor**, Department of Electrical and Computer Engineering at McMaster University and the Brain-Body Institute at St Joseph's Healthcare in Hamilton, Ontario, Canada.

Other Experience and Professional Memberships

1983-1985 **Lecturer in Physics**, New course on MRI, Case Western Reserve University, Cleveland, OH.
1992-1992 **Associate Editor**, IEEE for Transactions on Medical Physics.
1992-1994 **Chairman**, Liaison Committee at the Society for Magnetic Resonance Imaging (SMRI).
1992-1994 **Co-founder**, Joint Merger Evaluation Committee for the Society for Magnetic Resonance Imaging (SMRI) / International Society for Magnetic Resonance in Medicine (ISMRM).
1993 **Vice-President**, Interim Board at the Society of Magnetic Resonance Imaging (SMRI).
1993-1994 **President**, Society of Magnetic Resonance Imaging (SMRI).
2007-Present **Assoc Chair**, School of Medicine, Dept of Biomedical Eng, Wayne State University, Detroit, MI.

Honors

1989 Sylvia Sorken Greenfield Award for the best paper in Medical Physics
1992 Fellow of the Society Award for the Society of Magnetic Resonance Imaging

1994 Silver Medal Award, Society of Magnetic Resonance

1997 Poster Award at the 14th Annual Meeting, European Society for Magnetic Resonance in Medicine and Biology. J.R. Reichenbach, E.M. Haacke, B.C.P. Lee, Ch. Przetak, W.A. Kaiser

1998 Marie-Sklodowska-Curie Prize for Visualization of Cerebral Venous Structures Using High Resolution MRI by J.R. Reichenbach, L.R. Schad, M. Essig, E.M. Haacke, W.A. Kaiser

1999 Awarded the Visiting Professorship as the Roentgen Professor of Physics in Wuerzburg.

2000 Poster Prize of the XXVI Congress of the European Society of Neuroradiology 2000. J.R. Reichenbach, L. Jonetz-Mentzel, C. Fitzek, H.-J. Mentzel, E.M. Haacke, W.A. Kaiser.

2002 Scientific Exhibition Award ECR 2002 Cum Laude. J.R. Reichenbach, C. Fitzek, L. Jonetz-Mentzel, D. Sauner, H.-J. Mentzel, E.M. Haacke, W.A. Kaiser. European Congress of Radiology

2004 Gold Medal Award, International Society of Magnetic Resonance in Medicine

2006 Wayne State University, Office of the Vice President for Research, Research Mentors Award Program for New Faculty for mentoring of Dr. Yu-Chung Norman Cheng

2006 RSNA Educational Exhibit Award LL-NR4709 entitled "Susceptibility Weighted Imaging (SWI) of the Brain: Pictorial Review of the Technique, Anatomy, and Pathology" T. Hirai, MD, Kumamoto JAPAN; M. Akter; M. Kitajima, MD; T. Okuda, MD; E.M. Haacke, PhD; Y. Yamashita, MD

2008 Best Abstract Award "Improving the detection of diffue axonal injury by complementary use of advanced MRI" at the 6th North American Brain Injury (NABIS) Annual Conference. Z. Kou, R. Benson, R. Gattu, M. Haacke. The abstract presented our breakthrough on a complementary use of SWI and DTI techniques for injury detection.

2009 Regional Scholarship for Asia "Imaging the Vessel Wall in Major Peripheral Arteries using Susceptibility Weighted Imaging: Visualizing Calcifications" at the 12th Annual Society of Cardiovascular Magnetic Resonance (SCMR). Qi Yang, Kuncheng Li, Jiangtao Liu, S. Barnes, Z. Wu, J. Neelavalli, J. Hu, E.M. Haacke.

C. Selected peer-reviewed publications. (Selected from 218 peer-reviewed publications)

Most relevant to the current application

1. E.M. Haacke, F.H. Bearden, J.R. Clayton and N.R. Linga. Reduction of MR Imaging Time by the Hybrid Fast Scan Technique. Radiology **1986**;158:521-529.
2. E.M. Haacke, C.L. Filleti, R. Gattu, C. Ciulla, A.Al-Bashir, K. Suryanarayanan, M. Li, Z. Latif, Z. DelProposto, V. Sehgal, T. Li, V. Torquato, R. Kanaparti, J. Jiang, J. Neelavalli. New Algorithm for Quantifying Vascular Changes in Dynamic Contrast-Enhanced MRI Independent of Absolute *T*1 Values. MRM **2007** – 58:463-472.
3. Hillman GG, Singh-Gupta V, Zhang H, Al-Bashir AK, Katkuri Y, Li M, Yunker CK, Patel A, Abrams J, Haacke EM. DCE-MRI of vascular changes induced by sunitinib in papillary renal cell carcinoma xenograft tumors. Neoplasia **2009** - 11;910-920. PMCID: PMC2735805.
4. Yu Y., Q. Jiang , Y. Miao, J. Li , H. Wang, S. Bao, C. Wu, X. Wang., J. Zhu, Y. Zhong, EM Haacke, J. Hu. "Quantitative analysis of clinical dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) to evaluate treatment response in human breast cancer" is conditionally accepted by Radiology.

Additional recent publications of importance to the field (in chronological order)

1. M Haacke, S. Mittal, Z. Wu, J. Neelavalli, Y.C. Cheng. Susceptibility-weighted imaging: technical aspects and clinical applications, part 1. AJNR **2009** - 30:19-30.
2. Y.C. Cheng, J. Neelavalli, E.M. Haacke. Limitations of calculating field distributions and magnetic susceptibilities in MRI using a Fourier based method. Phys Med Biol. **2009** - 54:1169-1189.
3. S. Mittal, Z. Wu, J. Neelavalli, E.M. Haacke. Susceptibility-Weighted Imaging: Technical Aspects and Clinical Applications, Part 2. AJNR **2009** – 30:232-252.
4. E.M. Haacke, M. Makki, Y. Ge, M. Maheshwari, V. Sehgal, J. Hu, M. Selvan, Z. Wu, Z. Latif, Y. Xuan, O. Khan, J. Garbern. Characterizing Iron Deposition in Multiple Sclerosis Lesions Using Susceptibility Weighted Imaging. JMRI **2009** – 29;537-544. PMCID: PMC2650739.
5. S.R.S. Barnes and E.M. Haacke. Susceptibility Weighted Imaging: Clinical Angiographic Applications. MRI Clinical N Am **2009** - 17;47-61. PMCID: PMC2713115.
6. J. Neelavalli, Y-C.N. Cheng, J. Jiang, E.M. Haacke. Removing Background Phase Variations in Susceptibility Weighted Imaging Using a Fast, Forward-Field Calculation. JMRI **2009** – 29;937-948.

7. Y. Ge, V.M. Zohrabian, E-O. Osa, J. Xu, H. Jaggi, J. Herbert, E.M. Haacke, R.I. Grossman. Diminished visibility of cerebral venous vasculature in multiple sclerosis by susceptibility-weighted imaging at 3.0 T. JMRI **2009** – 29;1190-1194.
8. E.S. Manova, C.A. Habib, A.S. Boikov, M. Ayaz, A. Khan, W.M. Kirsch, D.K. Kido, E.M. Haacke. Characterizing the mesencephalon using susceptibility weighted imaging. AJNR **2009** - 30;569 –574.
9. Q. Yang, J. Liu, S.R.S. Barnes, Z. Wu, K. Li, J. Neelavalli, J. Hu, and E.M. Haacke. Imaging the Vessel Wall in Major Peripheral Arteries using Susceptibility Weighted Imaging: Visualizing Calcifications. JMRI **2009** - 30;357-365. PMCID: PMC2730889.
10. Chavhan, G.B., Babyn, P.S., Thomas, B., Shroff, M.M., Haacke, E.M. Principles, Techniques, and Applications of T2*-based MR Imaging and Its Special Applications1. RadioGraphics **2009**;29;1433-1449.
11. Cheng, Y-C N., Hsieh, C-Y, Neelavalli, J. and Haacke, E.M. Quantifying effective magnetic moments of narrow cylindrical objects in MRI. Phys. Med. Biol. **2009**-54;7025-7044.

C. Research Support.

H133G080064 (Hanks) 10/01/2008 – 09/30/2010 0.00 calendar
National Institute on Disability $593, 022
and Rehabilitation Research
Neuroanatomical Correlates of Positive Psychology Among People with Traumatic Brain Injury: A Biopsychosocial Model. *A Field Initiated Grant*
Goals: Improve our ability to identify individual characteristics and resources that can be used to facilitate well-being and recovery of function after TBI.

Guerbet (Haacke) 11/16/2009-08/31/2010 0.24 calendar
P904 Stroke Study $50,000
Goals: To further test a contrast agent on stroke animals to see if it will work sufficiently to indicate areas of angiogensis.

NSF 06-597 (Dong) 06/01/2008 – 05/31/2011 0.60 calendar
National Science Foundation $270,822
CRI:IAD Acquisition of Research Infrastructure for Knowledge-enhanced, Large-scale Learning of Multimodality Visual Data
Goals: Purchase a major piece of equipment for data storage.

2R01 HL062983-04A2 (Haacke) 09/01/2008 – 05/31/2011 2.40 calendar
National Institutes of Health $1,560,829
Susceptibility Weighted Imaging (SWI)
Goals: Continue the development of SWI to: a) make it more clinically viable by reducing phase processing artifacts; b) evaluate susceptibility itself by creating a susceptibility map of human tissue; c) study its role as a new MR angiographic method by simultaneously collecting MRA and SWI data; and d) speed up its acquisition time to less than 5 minutes for whole brain coverage, independent of any parallel imaging gain factor.

K08 MH079176A (Behen) 09/03/2007 – 07/31/2012 0.00 calendar
National Institutes of Health /NIMH $680,483
Structural and Functional Neural Correlates of Early Postnatal Deprivation
Goals: Evaluate the neuroanatomical correlates of early social deprivation (ESD) in human children using both state-of-the-art MRI and PET methods.

Master Research Agreement (Haacke) 07/01/2009 – 06/30/2012 0.60 calendar
Siemens Medical Solutions $300,000
Research Agreement
Goals: Collect clinical SWI data for trauma, stroke, and vascular disease.

R01 NS041922 (Juhasz)	07/01/2008 – 04/30/2013	0.96 calendar
National Institutes of Health/NINDS	$990,000	

Longitudinal neuroimaging in Sturge-Weber syndrome"
Goals: To study the effects of Sturge-Weber syndrome on the brain over time.

University Of Saskatchewan (Nichol)	11/1/2009 – 10/31/2014	0.24 calendar
CIHR Team in Synchrotron Medical Imaging	$337,300	

Goals: 1) map iron in fixed human brains to see changes in metal distribution associated with stroke and 2) Compare DCE MRI with SWI to better understand the etiology of vascular damage prior to the appearance of bleeds and quantify changes in elemental distribution associated with vascular permeability.

OVERLAP

No overlap for Dr. Haacke exists.

Principal Investigator/Program Director (Last, First, Middle): Kreipke, Christian, W

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Donald M. Kuhn	Professor
eRA COMMONS USER NAME	
aa3071	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Presbyterian College	BS	1972	Biopsychology
University of South Carolina	PhD	1976	Behavioral Pharmacology
Princeton University	Postdoc	1976-1977	Electrophysiology
National Institutes of Health	Postdoc	1977-1983	Biochemical Pharmacology

A. Personal Statement

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults. TBI results in 3 major pathologies: 1. Cerebral edema which leads to elevated ICP, 2. Diffuse axonal injury which brings about disruption of neural circuits underlying cognitive behavior, and 3. Alterations in the brain's microcirculation that cause persistent hypoperfusion and improper delivery of vital metabolites to neural tissue. While clinical trials aimed at the first two pathologies have been developed, to date none has addressed the third pathology, hypoperfusion following TBI. The present proposal uses a novel antagonist of endothelin-1 receptor A (ETrA), Clazosentan, to improve CBF and ultimately cognition. My laboratory has a long history of performing pharmacological studies and therefore I will aide Dr. Kreipke with his design of dosing and dosing regime for Clazosentan. Furthermore, this work will compliment our co-funded VA grant which combines our expertise to develop future therapeutics for TBI victims.

B. Positions and Honors

Positions and Employment

1983-1986-Chief, Section on Biochemical Pharmacology, National Heart Lung & Blood Institute, NIH
1985-1986-Alexander von Humboldt Fellow, Department of Neurochemistry, Goethe University, Frankfurt, Germany
1987-present- Professor, Department of Psychiatry and Behavioral Neurosciences, Center for Molecular Medicine and Genetics, and Institute for Chemical Toxicology, Wayne State University School of Medicine
1993-1994-Visiting Professor, Dept. Molecular Genetics and HHMI, Univ. Texas Southwestern Medical Center, Dallas, Texas (Sabbatical leave in Dr. T. Sudhof's lab)
1998-present- Research Career Scientist, John D. Dingell VA Medical Center, Detroit, MI

Other Experience and Professional Memberships

1994-1998 Member, NIDA-C (now NMB) Scientific Review Subcommittee
1998-2002 Member, MDCN-4 Scientific Review Subcommittee
1999- Member, Editorial Board Journal of Neurochemistry
1999- Ad hoc reviewer for MDCN-3, IFCN-7, Neurological Sciences & Disorders B, NIDA Cebra Program, and numerous SEPs for NIDA, NINDS, and NIMH
2001- National Scientific Advisory Council, American Federation for Aging Research
2004- Member, Neurobiology A Merit Review Subcommittee, Dept. Veterans Affairs
2006- Member, NMB Scientific Review Subcommittee

Honors

1985- Fellow, Alexander von Humboldt Foundation

C. Selected peer-reviewed publications (in chronological order)

(Publications selected more than 135 peer-reviewed publications and book chapters)

Wolf, W.A. and **Kuhn, D.M.** Molecular pharmacology of the neuronal serotonin transporter: Role of essential sulfhydryl groups in ligand binding and transport. J. Biol. Chem. 267, 20820-20825, 1992.

Kuhn, D.M. and Geddes, T.J. Peroxynitrite inactivates tryptophan hydroxylase via sulfhydryl oxidation: Coincident nitration of enzyme tyrosyl residues has minimal impact on catalytic activity. J. Biol. Chem. 274, 29726-29732, 1999.

Anastasiadis, P.Z., Jiang, H., Bezin, L., **Kuhn, D.M.**, and Levine, R.A. Tetrahydrobiopterin enhances apoptotic cell death following withdrawal of trophic support. J. Biol. Chem. 276, 9050-9058, 2001.

Kuhn, D.M. Dopamine and Its Modulation of Drug-Induced Neuronal Damage, E. Massaro (Ed.). In: Handbook of Neurotoxicology, Volume 2, Drugs of Abuse, Humana Press, pp 175-197, 2002.

Kuhn, D.M., Sadidi, M., Lu, X., Kreipke, C., Geddes, T., Borges, C., and Watson, J.T. Peroxynitrite-induced nitration of tyrosine hydroxylase: Identification of tyrosines 423, 428, and 432 as sites of modification by MALDI-TOF mass spectrometry and tyrosine-scanning mutagenesis. J. Biol. Chem., 277, 14336-14342, 2002.

Kuhn, D.M. and Geddes, T.J. Reduced nicotinamide nucleotides prevent nitration of tyrosine hydroxylase by peroxynitrite. Brain Research, 933, 85-89, 2002.

Thomas, D.M., Dowgiert, J., Geddes, T.J., Verbeem, D., Liu, X., and **Kuhn, D.M.** Microglial activation is a pharmacologically specific marker for the neurotoxic amphetamines. Neurosci. Lett., 367, 349-354, 2004.

Thomas, D.M. and **Kuhn, D.M.** Attenuated microglial activation mediates tolerance to the neurotoxic effects of methamphetamine. J. Neurochem., 92, 790-797, 2005.

Thomas, D.M., Francescutti-Verbeem, D.M., and **Kuhn, D.M.** Gene expression profile of activated microglia under conditions associated with dopamine neuronal damage. FASEB J (FJ Express Summary), 20, 515-517, 2006.

Kuhn, D.M., Sakowski, S.A., Geddes, T.J., Wilkerson, C., and Haycock, J.W. Phosphorylation and activation of tryptophan hydroxylase 2: Identification of serine-19 as the substrate site for calcium-dependent protein kinase II. J. Neurochem., 103, 1567-1573, 2007.

Thomas, D.M., Francescutti-Verbeem, D.M., and **Kuhn, D.M.** The newly synthesized pool of dopamine determines the severity of methamphetamine-induced neurotoxicity. J. Neurochem., 605-616, 2008.

Kuhn, D.M., Francescutti-Verbeem, D.M., and Thomas, D.M. Dopamine disposition in the presynaptic process regulates the severity of methamphetamine-induced neurotoxicity. Ann. N.Y. Acad. Sci., in press, 2008.

Kuhn, D.M., Francescutti-Verbeem, D.M., and Thomas, D.M. Dopamine disposition in the presynaptic process regulates the severity of methamphetamine-induced neurotoxicity. Ann. N.Y. Acad. Sci., 1139, 118-126, 2008.

Thomas, D.M., Francescutti-Verbeem, D.M., and **Kuhn, D.M.** Increases in cytoplasmic dopamine compromise the normal resistance of the nucleus accumbens to methamphetamine neurotoxicity. J. Neurochem., 109, 1745-1755, 2009.

Angoa-Pérez M, Kreipke CW, Thomas DM, Van Shura KE, Lyman M, McDonough JH, **Kuhn DM**. Soman Increases Neuronal COX-2 Levels: Possible Link between Seizures and Protracted Neuronal Damage. Neurotoxicology. 2010 Jul 2. [Epub ahead of print]

D. Research Support

Ongoing (Active) Research Support

NIH/NIDA 5 R01 DA10756 04/10/07-04/09/12

Neurotoxic Amphetamines, Radicals, and 5HT Neurons

The major goal of the study is to determine the mechanisms by which neurotoxic amphetamine-derived reactive oxygen and nitrogen species alter function of dopamine and serotonin neurons through their effects on important phenotypic marker proteins in these neuronal elements.

NIH/NIDA 1 RO1 DA017327 04/01/05 – 03/30/10
Methamphetamine Neurotoxicity and Microglial Activation
The goal of this project is to elucidate the role of microglia in the neurotoxic effects associated with methamphetamine and other neurotoxic amphetamines.
Role: PI

Department of Veterans Affairs Merit Award 03/15/07-03/14/11
Brain Injury by Blast Overpressure: Role of Microglial Activation
The goal of this project is to characterize microglial involvement in brain damage caused by blast overpressure. We have developed a model of blast overpressure, a form of traumatic brain injury, that allows testing of cultured cells and brain slices.
Role: PI

R01 NS064976-A2 Kreipke (PI) 11/01/09-10/31/14
NIH_NINDS
"Molecular Mechanisms of Enhanced Contractility following Traumatic Brain Injury: towards a clinical trial" (Investigates the mechanism by which endothelin receptor antagonists may be useful in the treatment of cognitive deficits following TBI).
Role: Co-I

VARR&D 1I01RX000224-01 Kreipke (PI) 11/01/09-10/31/12
"Poly-trauma following brain injury: towards a combinatorial therapy" (Investigates the effects of multiple pathologies associated with traumatic brain injury on histopathological and behavioral outcome).
Role: Co-I

Projects completed in the past 3 years

NIH/NIDA 1 K05 DA14692 10/05/02-12/04/07
Molecular Biology of Drug Abuse
This is a senior scientist career development award.
Role: PI

NIH/NIDA 1 T32 DA07310 07/01/00-06/30/06
Neuroscience Training in Drug Abuse
This is a training grant that supports two predoctoral and two postdoctoral fellows. This training program is in hiatus temporarily. Our department experienced some significant changes in faculty re-assignment to other academic units, and several other key investigators on the T32 have left Wayne State. Therefore, we are re-configuring this training program as the Translational Neuroscience Program to reflect more accurately the current mentoring and research expertise of our departmental faculty.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Susan Sheps Margulies, Ph.D.	Professor in Bioengineering
eRA COMMONS USER NAME (credential, e.g., agency login) MARGULIE	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Princeton University, Princeton, NJ	BSE	1982	Mech. & Aerosp. Eng'g
University of PA, Philadelphia, PA	MSE	1983	Bioengineering
University of PA, Philadelphia, PA	PhD	1987	Bioengineering
Mayo Graduate School of Medicine	Res. Fellow	1987-89	Thoracic Diseases

A. Personal Statement

Dr. Margulies, a Professor in Bioengineering, is an investigator on the project, and will oversee all aspects of the pig behavioral assessments in proposed research plan. Dr. Margulies has over 25 years of experience in the area of traumatic brain injury research; many recent publications focus on the biomechanics of traumatic brain injury in adults and children, and assessments of cognition, memory and behavior in piglets.

B. Positions and Honors

Positions and Employment

1989-1990 Senior Res Fellow, , Mayo Med. School, Mayo Clinic, Rochester, MN
1989-1990 Instructor, Dept. of Physiology & Biophysics, Mayo Medical School, Mayo Clinic, Rochester,MN
1990-1992 Research Associate, Thoracic Diseases Research Unit, Mayo Clinic, Rochester, MN
1990-1993 Assistant Professor, Dept. of Physiology & Biophysics, Mayo Medical School, Rochester, MN
1992-1993 Associate Consultant, Thoracic Diseases Research Unit, Mayo Clinic, Rochester, MN
1993-1998 Assistant Professor, Department of Bioengineering, University of PA, Philadelphia, PA
1998-2004 Associate Professor, Department of Bioengineering, University of PA, Philadelphia, PA
2004-present Professor Dept. of Neurosurgery, Univ. of PA, Philadelphia, PA
2004-present Professor, Department of Bioengineering, University of PA, Philadelphia, PA
2007-present Graduate Group Chair, Bioengineering University of PA, Philadelphia, PA

Federal Advisory Committees:

1997-2002 Member, NSF Review Panel - Bioengineering Grants, CAREER Awards, Graduate Fellowships
2002 Member, NSF Committee of Visitors -Review of Bioengineering (BES) Division
2000-2003 Member, CDC Injury Research Grant Review Committee
2001 Member, NIH Study Section ZRG1 SSS-3 (03)
2002, 2008 Member NIH NHLBI PPG Study Section
2003-4 Member (ad hoc), NIH RESP and RIBT Study Sections
2008-pres Member (standing), NIH RIBT Study Section
2007- 2010 Member, New Jersey Commission on Brain Injury Research Scientific Review Committee

Honors:

1982 Summa Cum Laude, Princeton University; 1982 Tau Beta Pi, Sigma Xi Honor Societies; 1992 Whitaker Foundation Young Investigator Award; 1996 S. Reid Warren Award for Distinguished Teaching; 1997 NSF Career Award; 2001 American Society of Mechanical Engineers Richard Skalak Best Paper Award; 2006 Fellow of the American Institute of Medical and Biological Engineering; 2007 Assoc of Women in Science

Elizabeth W. Bingham Award for mentoring; 2009 Ford Motor Company Award for Faculty Advising; 2009 Fellow of the American Society for Mechanical Engineers; Fellow of the Biomedical Engineering Society.

C. Selected peer-reviewed publications (in chronological order).
(Publications selected from 92 peer-reviewed publications)

1. Duhaime AC, Gennarelli TA, Thibault LE, Bruce DA, **Margulies SS**, Wiser R. The shaken baby syndrome - a clinical, pathological, and biomechanical study. J Neurosurg 1987; 66:409-415. PMID: 3819836
2. **Margulies SS**, Thibault LE. An analytical model of diffuse brain injury. J Biomech Eng 1989; 111:241-249. PMID: 2779190
3. **Margulies SS**, Thibault LE. A proposed tolerance criterion for diffuse axonal injury in man. J Biomech 1992; 25:917-923. PMID: 1639835
4. Duhaime AC, Epplely M, **Margulies SS**, Heher KL, Bartlett SP. Crush injuries to the head in children. Neurosurgery 1995; 37:401-407. PMID: 7501102
5. **Margulies SS** and Thibault KL Infant skull and suture properties: measurements and implications for mechanisms of pediatric brain injury. J Biomech Eng.122:364-371, 2000. PMID: 11036559
6. Duhaime AC, **Margulies SS,** Durham SR O'Rourke MM, Golden JA ,Marwaha S, Raghupathi R. Maturation-dependent response of the piglet brain to scaled cortical impact J Neurosurgery. 93(3):455-62, 2000. PMID: 10969944
7. Prange MT and **Margulies SS**. Regional, directional and age-dependent properties of brain undergoing large deformation. J Biomed Eng. 2002; 124:244-252. PMID: 12002135
8. Raghupathi, R and **Margulies SS**. Traumatic axonal injury after closed head injury in the neonatal pig. J Neurotrauma 2002; 19:843-853. PMID: 12184854
9. Prange MT, Coats B, Duhaime AC, and **Margulies SS**. Anthropomorphic simulations of falls shakes, and inflicted impacts for infants. J Neurosurg. 2003, 99: 143-150. PMID: 12854757
10. Raghupathi R, Mehr MF, Helfaer MA, and **Margulies SS.** Traumatic Axonal Injury is Exacerbated following Repetitive Closed Head Injury in the Neonatal Pig. J Neurotrauma 2004; 21:307-316. PMID: 15115605
11. Gefen A and **Margulies SS**. Are in vivo and in situ brain tissues mechanically similar? J Biomech 2004 37:1339-1352. PMID: 15275841
12. Ji S, Dougherty L, **Margulies SS.** In vivo measurements of human brain displacement. Stapp Car Crash Journal, 2004. 48:227-37. PMID: 17230268
13. Zhu Q, Prange M., **Margulies SS.** Predicting Unconsciousness From a Pediatric Brain Injury Threshold. Developmental. Neurosci (Invited) 2006:28:388-395. PMID: 16943662
14. Coats BS and **Margulies S**S. Material Properties of Human Infant Skull and Suture at High Rates. J Neurotrauma 2006: 23(8):1222-1232. PMID: 16928180
15. Ichord R., Naim M., Pollack A., Ibrahim N., Christian C, and **Margulies SS**. Hypoxic-ischemic Injury Complicates Inflicted and Accidental Traumatic Brain Injury in Young Children: Role of Diffusion-Weighted Imaging. J Neurotrauma (Invited) 2007: 24:106-118. PMID: 17263674
16. Friess SH, Ichord R, Owens K, Ralston J, Overall K, Smith C, Helfaer M, and **Margulies SS**. Neurobehavioral Functional Deficits Following Closed Head Injury in the Neonatal Pig. Exper Neurol 2007: 204:234-243. PMID: 17174304
17. Coats B and **Margulies SS**. Potential for Head Injuries in Infants from Low Height Falls. Journal of Neurosurgery - Pediatrics 2008: Nov, 2(5):321-30. PMID: 18976102
18. **Margulies SS**, Hicks, R and the Combination Therapies for Traumatic Brain Injury Workshop Leaders. Combination Therapies for Traumatic Brain Injury – Prospective Considerations. J. Neurotrauma 2009: 26:925-939. PMID: 19331514

19. Friess SH, Ichord R, Owens K, Ralston J, Ryall K, Helfaer M, Smith C, and **Margulies SS**. Repeated traumatic brain injury affects composite cognitive function in piglets. Journal of Neurotrauma 2009: 26:1111-1121. PMID: 19275468

20. Ibrahim, NG, Natesh R, Szczesny SE, Ryall K, Eucker SA, Coats B, and **Margulies SS** – In Situ Deformations in the Immature Brain During Rapid Rotations. J Biomech Eng 2010, 132: (in press).

D. Current Research Support:

R01-HL-57204 Margulies (PI) 7/01/97 – 4/30/11
National Institutes of Health/NHLBI
Mechanical Injury of the Alveolar Epithelium
In this competitive renewal our major objective is to determine specific stretch-induced mechanical and molecular signals that modulate alveolar epithelial permeability during clinically relevant conditions - including chronic continuous cycling and ARDS
Role: PI

R01-NS-39679 Margulies (PI) 12/01/99 – 3/31/12
NIH- National Institute for Neurological Disorders and Stroke
Biomechanics of Pediatric Head Injury
In this competitive renewal our major objective is to determine mechanisms of primary and secondary brain injury in children, with an emphasis on both mild and severe injuries. The influences of rotation direction, hypoxia/ischemia, and repeated injuries will be studied using animal experiments and computational models.
Role: PI

R01-NS-039679-08S1 Margulies(PI) 09/01/09 – 08/31/11
National Institutes of Health
Biomechanics of Pediatric Head Injury
In this supplemental research plan we will extend the existing goals of the grant by adding the following two adjunct studies to accelerate the pace of research, enhance potential for determining mechanisms oft traumatic brain injury in infants, and developing new methods for injury diagnosis.

R01-CE-001445 Margulies (PI) 9/30/08 – 9/30/11
Center for Disease Control, NCIPC
Development and Validation of a Diagnostic Tool for Infant Head Injuries from Falls
Biomechanical loads from surrogate experiments, pediatric large animal TBI data, and a computational model will be combined to create a predictive tool to determine the plausibility of skull fracture and extra-axial hemorrhages in infants following low height falls. Validated with real-word clinical data, this biomechanical data will advance the understanding of injury thresholds in common non- inflicted scenarios that will ultimately improve the accuracy in detection of inflicted and non-inflicted head trauma.
Role: PI

DTNH22-07-H-00088 Margulies (PI) 6/1/07-5/30/10
Department of Transportation - NHTSA
Brain Injury Criteria for 6 to 10 year old Children
This new project utilizes human accident reconstructions, 2-month old porcine inertial brain injury studies, and finite element modeling to identify injury thresholds for school-age children.
Role: PI

R01-NS-055951 Bayly (PI) 1/01/07 – 12/30/11
National Institutes of Health via subcontract from Washington University
In vivo Measurement of Brain Biomechanics
This project involves the use of brain-skull motion studies and brain property elastography studies in the measurement of the biomechanics of the brain.

Role: Investigator

K08-NS064051 (CHOP PI, Friess; UP PI Margulies) 04/01/10 – 03/30/15
National Institutes of Health via subcontract from Children's Hospital of Philadelphia
Modulating secondary damage following traumatic brain injury in the child
The goal of this project to determine the contributions of cerebral perfusion pressure and endothelin-1 to cerebral hypoperfusion after pediatric traumatic brain injury, and to develop early interventions that will improve functional outcomes.
Role: Mentor and Subcontract PI

Pending Research Support:
U01-NS-069545 Margulies (PI) 12/01/10 – 11/30/15 NINDS FUNDING APPROVED 9/10
NIH - National Institute for Neurological Disorders and Stroke
Preclinical Cyclosporin A Therapy Trials for Pediatric TBI
The objective of this study is to conduct an immature large animal preclinical study of TBI to evaluate effectiveness of cyclosporine A to improve functional outcomes after pediatric TBI.

U01 Kreipke/Armstead (PI) 12/01/10 – 11/30/15
NIH

BIOGRAPHICAL SKETCH

NAME **Mueller, Patrick J.**	POSITION TITLE Assistant Professor
eRA COMMONS USER NAME MUELLERP	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Blackburn University, Carlinville, Illinois	B.A.	1990	Biology/Chemistry
St. Louis University, St. Louis, Missouri	Ph.D.	1996	Pharmacol. & Physiol.
Medical College of Wisconsin, Milwaukee, WI	Postdoc	1995-1997	Exerc Phys./Neural Ctrl.
University of Missouri, Columbia, MO	Postdoc	1997-2001	Neural Ctrl. Circulation

A. Personal Statement

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults. TBI results in 3 major pathologies: 1. Cerebral edema which leads to elevated ICP, 2. Diffuse axonal injury which brings about disruption of neural circuits underlying cognitive behavior, and 3. Alterations in the brain's microcirculation that cause persistent hypoperfusion and improper delivery of vital metabolites to neural tissue. While clinical trials aimed at the first two pathologies have been developed, to date none has addressed the third pathology, hypoperfusion following TBI. The present proposal uses a novel antagonist of Endothelin-1, Clazosentan, to improve CBF and ultimately cognition. My laboratory has extensive experience in assessing hemodynamics under various conditions. Therefore, I will aide in the interpretation of all hemodynamic data. Further, if necessary, I will contribute my time to aide Dr. Kreipke in assessing whether his drug, Clazosentan, has effects on peripheral perfusion.

B. Positions and Honors.

Positions and Employment

1990-1995	Graduate Trainee, St. Louis University Health Sciences Center
1995-1997	Postdoctoral Fellow, Medical College of Wisconsin
1997-2001	Postdoctoral Fellow, University of Missouri-Columbia (MU)
2001-2/2007	Research Assistant Professor, Dalton Cardiovascular Research Center, MU
2003-2/2007	Research Investigator, Dalton Cardiovascular Research Center, MU
2003-2/2007	Adjunct Research Assistant Professor, Department of Biomedical Sciences, MU
3/2007-	Assistant Professor, Department of Physiology, Wayne State University School of Medicine
8/2007-	Graduate Faculty, Wayne State University School of Medicine

Honors

1989	Bonnie Keith Albracht Scholarship, Blackburn College, Carlinville IL
1989	C.H.C. Anderson Prize, Blackburn College, Carlinville IL
1989	Drew Thurston Memorial Award, Blackburn College, Carlinville IL
2001	Caroline tum Suden/Frances A. Hellebrandt Professional Opportunity Award, American Physiological Society, Experimental Biology Meeting
2001	Michael J. Brody Young Investigator Award, APS Neural Control and Autonomic Regulation Section, Experimental Biology Meeting
2001	Phi Zeta Research Day, 1st Place Oral Presentation, Advanced Graduate Students and

	Postdocs
2005	Research Recognition Award, APS Neural Control and Autonomic Regulation Section, Experimental Biology Meeting
2006	Research Career Enhancement Award, American Physiological Society Host Laboratory: Patrice Guyenet, Ph.D., University of Virginia
2007	Outstanding Poster picked for Oral Presentation FASEB Summer Research Conference: Sydney, Australia Neural Mechanisms in Cardiovascular Regulation
2009	New Investigator Award, APS Neural Control and Autonomic Regulation Section, Experimental Biology Meeting
2009	Travel Award and Invited Speaker, International Society of Autonomic Neuroscience Satellite Meeting "Autonomic Adjustments to Environmental Challenges" Newcastle, Australia

Other Experience and Professional Memberships

Societies: American Physiological Society, Society for Neuroscience, American College of Sports Medicine

Reviewer: Am J Physiol: Heart Circ Physiol; Am J Physiol: Reg Integr Physiol; Autonom Neurosci: Basic and Clin; J Appl Physiol; Med Sci Sports & Exercise, Hypertension, Exp Physiol, BMC Neurosci. Univ of Florida Mock Grant Review (03/2004); AHA National Consortium Peer Review Committee (10/07).

Related Activities: Faculty Grant Writing Institute-University of Missouri-Columbia (05/06); Lecturer, Univ. of Missouri-Columbia, Biomed. Sci. Course VBSCI9467 "Neural Control of the Circulation" (Spring '02,'04,'06); Instructor, Central Neural Control of the Circulation, The American Physiological Society Latin American Initiative, Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo-Brazil (08/04); Poster Judge, Cardiovascular Day, Univ of Missouri (02/04), Minority Research Day, WSU (08/07) ; Architect Advisory Committee-Dalton Cardiovascular Research Center Expansion and Renovation Project (2001-2003); APS Committee Member, Neural Control and Autonomic Regulation Steering Committee, Member in Training (2001-2002); Lecturer, Wayne State University School of Medicine, Dept of Physiol PSL7600 "Advanced Cardiovascular Physiology" (Fall 2007-09); Laboratory Instructor, Medical Physiology, Wayne State University School of Medicine (Fall 2007-09).

C. Peer-reviewed Publications (selected from 26 peer reviewed works and 2 book chapters) .

1. **Mueller, P.J**. and Knuepfer, M.M. Coronary vascular effects of cocaine in rats. J Pharmacol Exp Ther 268: 97-103, 1994. PMID: 8301600
2. **Mueller, P.J.**, Gan, Q. and Knuepfer, M.M. Ethanol alters hemodynamic responses to cocaine in rats. *Drug Alcohol Depend* 48:17-24, 1997. PMID: 9330917.
3. Buckwalter, J.B., **Mueller, P.J.** and Clifford, P.S. Autonomic control of skeletal muscle vasodilation during exercise. *J Appl Physiol* 83 (6): 2037-2042, 1997. PMID: 9390978. http://jap.physiology.org/cgi/reprint/83/6/2037
4. Buckwalter, J.B., **Mueller, P.J.** and Clifford, P.S. Sympathetic vasoconstriction to active skeletal muscles during dynamic exercise. *J Appl Physiol* 83 (5):1575-80, 1997. PMID: 9375322. http://jap.physiology.org/cgi/reprint/83/5/1575
5. Buckwalter, J.B., Ruble, S.B., **Mueller, P.J.** and Clifford, P.S. Skeletal muscle vasodilation at the onset of exercise. *J Appl Physiol* 85 (5): 1649-1654, 1998. PMID: 9804565. http://jap.physiology.org/cgi/reprint/85/5/1649
6. **Mueller, P.J.**, O'Hagan, K.P., Skogg, K.A., Buckwalter, J.B. and Clifford, P.S. Renal hemodynamic responses to dynamic exercise in rabbits. *J Appl Physiol* 85 (5): 1605-1614, 1998. PMID: 9804559. http://jap.physiology.org/cgi/reprint/85/5/1605
7. Knuepfer, M.M., Gan, Q. and **Mueller, P.J.** Mechanisms of hemodynamic responses to cocaine in conscious rats. *J Cardiovasc Pharmacol* 31:391-399, 1998. PMID: 9514184.
8. **Mueller, P.J.** and Hasser, E.M. Enhanced sympathoinhibitory response to volume expansion in conscious hindlimb unloaded rats. *J. Appl. Physiol.* 94: 1806-1812, 2003. PMID: 12533501. http://jap.physiology.org/cgi/reprint/94/5/1806

9. **Mueller, P.J.**, Buckwalter, J.B. and Clifford, P.S. Tracheal tone and the role of ionotropic glutamate receptors in the nucleus ambiguus. *Brain Research* 1021: 54-62, 2004. PMID: 15328031.
10. **Mueller, P.J.**, Foley, C.M., and Hasser, E.M. Hindlimb unloading alters nitric oxide and autonomic control of resting arterial pressure in conscious rats. *Am. J. Physiol. Reg. Integr. Comp. Physiol.* 289: R140-R147, 2005. PMID: 15761183. http://ajpregu.physiology.org/cgi/reprint/289/1/R140
11. **Mueller, P.J.**, Sullivan, M.J., Grindstaff, R.R., Cunningham, T.J. and Hasser, E.M. Regulation of plasma vasopressin and renin activity in conscious hindlimb-unloaded rats. *Am. J. Physiol. Reg. Integr. Comp. Physiol.* 291: R46-R52, 2006. PMID: 16469838. http://ajpregu.physiology.org/cgi/reprint/291/1/R46
12. **Mueller, P.J.** Exercise training and sympathetic nervous system activity: Evidence for physical activity dependent plasticity. *J. Clin. Exp. Pharmacol. Physiol.* 34(4):377-84, 2007. PMID: 17324153.
13. **Mueller, P.J.** Influence of sedentary versus physically active conditions on regulation of plasma renin activity and vasopressin. *Am. J. Physiol. Reg. Integr. Comp. Physiol.* 295: R727-R732, 2008. PMID: 18509102. http://ajpregu.physiology.org/cgi/reprint/295/3/R727
14. Heesch, C.M., Foley, C.M., **Mueller, P.J.**, Hasser, E.M. and Patel, K.P. Nitric oxide synthase activity and expression are decreased in the paraventricular nucleus of pregnant rats. *Brain Res* 1251:140-50, 2009. PMID: 19041855
15. Austgen, J.R., Fong, A.Y., Foley, C.M., **Mueller, P.J.**, Heesch, C.M. and Hasser, E.M. Expression of group I metabotropic glutamate receptors on phenotypically different cells within the nucleus of the solitary tract in the rat. *Neuroscience* 159: 701-716, 2009. PMID: 19013221

D. Research Support

Ongoing Research Support

HL089364 — Mueller (PI) — 08/10/07-07/31/10
R21, National Institutes of Health
"Physical Activity Dependent Plasticity in Central Sympathetic Nervous System Regulation"
Role: PI

American Heart Association, Predoctoral Fellowship — Mischel (PI) — 07/09-06/11
"NMDA Receptor Neuroplasticity in the RVLM Following Imposition of Sedentary Conditions."
Role: Sponsor

R01, National Institutes of Health, — Kreipke (PI) — 07/01/09-06/30/14
"Molecular Basis of Enhanced Contractility after Traumatic Brain Injury: Towards a clinical Trial"
Role: Co-Investigator (10%)

New Faculty Startup Funds — Mueller (PI) — 03/01/07-Present
Wayne State University School of Medicine

Pending Research Support

R01, National Institutes of Health — Mueller (PI) — 07/01/10-06/30/15 (Pending)
"Inactivity and Enhanced Sympathoexcitation: Role of Neuroplasticity in the RVLM"
Role: PI

Research Support Completed During the Last Three Years

0650161Z* Mueller (PI) — 01/01/06-12/31/08*
Grant In Aid, American Heart Association, Heartland Affiliate
"Central Control of Sympathetic Outflow Following Exercise Training"

The major goals of this project are to 1) Examine the effect of ExTr on activation of SNS activity and spinally projecting RVLM neurons. 2) Examine the effect of ExTr on regulation of SNS outflow by altering tonic excitatory and inhibitory neurotransmission in the RVLM.
Role: PI

*Transferred to Wayne State University School of Medicine from University of Missouri 03-01-07.

HL089364 Mueller (PI) 08/10/07-05/19/08
R21, National Institutes of Health Supplement 2
"Physical Activity Dependent Plasticity in Central Sympathetic Nervous System Regulation"
This supplement supported a minority undergraduate student who worked in my laboratory - Jason Franco

HL089364 Mueller (PI) 08/10/07-07/31/09
R21, National Institutes of Health (HL089364) Supplement 1
"Physical Activity Dependent Plasticity in Central Sympathetic Nervous System Regulation"
This supplement supported a minority undergraduate student who worked in my laboratory - Janet Adedokun

Principal Investigator/Program Director (Last, First, Middle): Kreipke, Christian, W

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Jose A Rafols	POSITION TITLE Professor
eRA COMMONS USER NAME JOSERAFOLS	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Illinois Benedictine, Lisle, IL	B.S.	1965	Biology
University of Kansas, Kansas City, KS	Ph.D.	1969	Anatomy
S. Ramon Y Cajal Institute, CSIC, Madrid, Spain	Post Doc	1970	Neuroanatomy

A. Personal Statement

Throughout my nearly 35 years in science, my laboratory has centered on determining the mechanisms of plasticity following both ischemic events and traumatic brain injury. Further I have extensive experience in measuring spine density as related to a measure of plasticity. I have mentored 10 predoctoral and 5 postdoctoral students/fellows and therefore will make my expertise and training efforts available to the candidate as needed.

B. Positions and Honors

Positions and Employment

1969-1970	Instructor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1970	NIH Postdoctoral trainee at S. Ramon Y Cajal Institute, CSIC, Madrid, Spain
1971-1973	Asst. Professor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1973-1989	Assoc. Professor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1989-present	Professor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1994-present	Dir., Morphology and Imaging Core, Neurotrauma Center, Wayne State University, School of Medicine

Honors

DHHS/PHS/NIH Study Section Member (full member), Neurological Disorder Program Project Review A Committee (NSP-term) 7/1/90-6/30/94.
Chairman, Site visit, The Johns Hopkins University, Baltimore, MD; "Disorders of aging neuro-transmitter systems and neurotrophins", December 15-17, 1991.
Member, National Institutes of Health Reviewers Reserve (NRR), for term 7/1/94-6/30/98.
Member, American Heart Association National Study Committee, Brain Review Committee, for term 7/96-6/99.

C. Peer-reviewed publications

1. Kreipke CW, Morgan N, Petrov T, Rafols J. 2006. Calponin and caldesmon cellular domains in reacting microvessels following traumatic brain injury. Microvascular Research 71:197-204.
2. Shen Y, Kou Z, Kreipke CW, Petrov T, Hu J, Haacke EM. 2007. In vivo measurement of tissue damage, oxygen saturation changes and blood flow changes after experimental traumatic brain injury in rats using susceptibility-weighted imaging. Magn Reson Imaging 25:219-227.

3. Kreipke CW, Morgan R, Petrov T, Rafols JA. 2007. Subcellular Redistribution of Calponin Underlies Sustained Vascular Contractility Following Traumatic Brain Injury. Neurological Research 29:604-609.
4. Kallukuri S, Kreipke CW, Rossi N., Rafols JA, Petrov T. 2007. Spatial alterations in endothelin receptor expression are temporally associated with the altered microcirculation after brain trauma. Neurological Research 29:362-368.
5. Kreipke CW, Morgan R, Roberts G, Bagchi M, Rafols JA. 2007. Calponin phosphorylation in cerebral cortex microvessels mediates sustained vasoconstriction after brain trauma. Neurological Research 29:369-374.
6. Kreipke CW, Petrov T, Rafols JA. 2007. Endothelin A receptor antagonism blocks calponin phosphorylation following brain trauma. J Cereb Blood Flow and Metab, 26, S191.
7. Kreipke CW, Schafer PC, Rafols JA. 2008. Endothelin receptor A antagonism ameliorates hypoperfusion and enhances cognitive outcome following traumatic brain injury. Brain Injury 22:S43.
8. Rafols JA, Kreipke CW, Kallakuri S. 2008. Upregulation of endothelin-1 receptors in neurons and brain microvessels coincides temporally with a dysfunctional microcirculation after traumatic brain injury. Brain Injury 22:S44.
9. Kreipke CW, Rafols JA. 2009. Calponin control of cerebrovascular reactivity: Therapeutic implications in brain trauma. J Cell Mol Med 13(2):262-9.
10. Ding JY, Kreipke CW, Speirs S, Schafer PC, Schafer S, Rafols JA. 2009. Hypoxia inducible factor-1□ signaling in aquaporin upregulation after traumatic brain injury. Neuros Lett. 453(1):68-72.
11. Ding JY, Kreipke CW, Speirs S, Schafer PC, Schafer S, Rafols JA. 2009. Synapse Loss Regulated by Matrix Metalloproteinases in Traumatic Brain Injury Is Associated with Hypoxia-Inducible Factor-1α Expression. Brain Research 1268:125-34.
12. Kreipke CW, Schafer PC, Rossi NF, Rafols JA. 2009 (Epub ahead of press). Differential affects of Endothelin receptor-A and B antagonism on hypoperfusion following traumatic brain injury (TBI). Neurological Research.
13. Kallakuri S, Kreipke CW, Schafer PC, Schafer SM, Rafols JA. (in press) Brain cellular localization of endothelin receptor A and B in a rodent model of diffuse brain injury. Neuroscience.
14. Dore-Duffy P, Ding Y, Zhan P, Schafer S, Fronczak M, Rafols JA, Kreipke CW. (in press) Endothelin receptor expression following ETrA antagonist treatment. Neurological Reseach.
15. Reynolds CA, Kallakuri S, Schafer S, Kreipke CW, Rafols JA. (in press) Endothelin receptor A antagonism reduces the extent of diffuse axonal injury in a rodent model of traumatic brain injury. Neurological Research.

D. Research Support

Ongoing Research Support

R01 NS064976-A2 Kreipke (PI) 11/01/09-10/31/14
NIH_NINDS
Role: CO-I
"Molecular Mechanisms of Enhanced Contractility following Traumatic Brain Injury: towards a clinical trial" (Investigates the mechanism by which endothelin receptor antagonists may be useful in the treatment of cognitive deficits following TBI).

VARR&D 1I01RX000224-01 Kreipke (PI) 11/01/09-10/31/12
Role: CO-I
"Poly-trauma following brain injury: towards a combinatorial therapy" (Investigates the effects of multiple pathologies associated with traumatic brain injury on histopathological and behavioral outcome).

VA RR & D Award Rossi/Kreipke (PI) 04/01/08-12/31/11
VA Rehabilitation

Role: CO-I

"Conditioning, microvascular tone & rehabilitation post brain trauma" (Investigates the role of exercise in the control of microcirculation in a rat model of traumatic brain injury).

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Smith, Douglas H.	Robert A. Groff Professor of Neurosurgery
eRA COMMONS USER NAME (credential, e.g., agency login) smithd	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Connecticut	B.S.	05/1981	Biology
University of Noreste	M.D.	05/1986	Medicine
University of Connecticut Health Center	Fellow	1986-1988	Biochemistry
University of Connecticut Health Center	Fellow	1988-1990	Neurotrauma

A. Personal Statement

Dr. Smith has over 18 years experience with brain injury research and has collaborated with the PI, Dr. Armstead, over several years. In particular, Dr. Smith's group has extensive expertise with traumatic brain injury in swine, examining histopathological outcome, as is proposed in the current application.

B. Positions and Honors

Positions and Employment

1986-88	Postdoctoral Fellow in Protein Chemistry and Molecular Biology, Dept. of Biochemistry, University of Connecticut Health Center, Farmington, CT
1989-90	Postdoctoral Fellow in Neurotrauma and Neuropharmacology, Dept. of Surgery, University of Connecticut Health Center, Farmington, CT
1991-92	Assistant Professor, Surgical Research Center, Dept. of Surgery, Univ. of Conn. Health Center
1992-97	Assistant Professor, Dept. of Neurosurgery, University of Pennsylvania, Philadelphia, PA
1995-01	Associate Director, Center for Brain Injury and Repair, University of Penn., Philadelphia, PA
1998-03	Associate Professor, Department of Neurosurgery, Univ. of Pennsylvania, Philadelphia, PA
2004-	Professor, Department of Neurosurgery, University of Pennsylvania, Philadelphia, PA
2004-	Director, Center for Brain Injury and Repair, University of Pennsylvania, Philadelphia, PA
2009-	Vice-Chairman for Research, Department of Neurosurgery, University of Pennsylvania, Philadelphia, PA
2009-	Robert A. Groff Professor of Neurosurgery, University of Pennsylvania School of Medicine

Honors

1992	University of Connecticut Health Center Research Advisory Committee Faculty Award
1993	Young Scientist Award, 2nd International Neurotrauma Symposium, Glasgow, Scotland
1994	Brain Trauma Foundation Research Award
1995-	Member, Editorial Board, Journal of Neurotrauma
2000-03	Member, Brain Disorders and Cognitive Neuroscience-3 (BDCN-3) Study Section, National Institutes of Health
2003-04	Vice-President, National Neurotrauma Society
2006-09	Councilor, National Neurotrauma Society
2009-	Robert A. Groff Endowed Professor of Neurosurgery, University of Pennsylvania, Philadelphia, PA

C. Selected Peer-reviewed Publications (Selected from over 140 peer-reviewed publications)

5 Most Relevant Publications

Johnson VE, Stewart W, Smith DH. Traumatic brain injury and amyloid-beta pathology: a link to Alzheimer's disease? *Nat Rev Neurosci.* 2010 [e-pub ahead of print). PMID: 20216546

Haacke EM, Duhaime AC, Gean AD, Riedy G, Wintermark M, Mukherjee P, Brody DL, DeGraba T, Duncan TD, Elovic E, Hurley R, Latour L, Smirniotopoulos JG, Smith DH. Common Data Elements in Radiologic Imaging of Traumatic Brain Injury. J of Magnetic Resonance Imaging. 32: 516-543, 2010. PMID: 20815050

Tang-Schomer MD, Patel AR, Baas PW, Smith DH: Mechanical Breaking of Microtubules in Axons During Dynamic Stretch Injury Underlies Delayed Elasticity, Microtubule Disassembly and Axon Degeneration. FASEB. 24(5): 1401-1410, May 2010. PMID: 20019243. PMCID: PMC2879950.

Armstead WM, Ganguly K, Kiessling JW, Riley J, Chen XH, Smith DH, Stein SC, Higazi AAR, Cines DB, Bdeir K, Zaitsev S, Muzykantov VR. Signaling, delivery and age as emerging issues in the benefit/risk ratio outcome of tPA for treatment of CNS ischemic disorders. *J Neurochem*, 113: 303-312, 2010. PMID: 20405577.

Armstead WM, Ganguly K, Kiessling JW, Chen XH, Smith DH, Higazi AR, Cines DB, Bdeir K, Zaitsey S, Muzykantoy VR. RBC-coupled tPA prevents impairment of cerebral vasodilatory responses and tissue injury in pediatric cerebral hypoxia/ischemia through inhibition of ERK MAPK activation. *JCBFM.* 29(8):1463-1474, 2009. PMID: 19436314. PMCID: PMC2719676.

10 Additional Publications

Uryu K, Chen X-H, Martinez D, Browne KD, Johnson VE, Graham DI, Lee VM-Y, Trojanowski JQ, Smith DH. Multiple proteins implicated in neurodegenerative diseases accumulate in axons after brain trauma in Humans *Exp. Neurology* 208(2): 185-92, 2007. PMID: 17826768

Zhang J, Groff R, Chen X-H, Browne KD, Huang J, Schwartz ED, Meaney DF, Johnson VE, Stein SC, RojkjaerR, Smith DH. Hemostatic and neuroprotective effects of human recombinant activated factor VII therapy after traumatic brain injury in pigs. *Exp. Neurol.*, 210(2):645-55, 2008. PMID: 18291370

Chen X-H, Johnson VE, Uryu K, Trojanowski JQ, Smith DH. A lack of amyloid β plaques despite persistent accumulation of amyloid β in axons of long-term survivors of traumatic brain injury. *Brain Pathology* 19(2):2214-23, 2008. PMID: 18492093

Johnson VE, Stewart W, Stewart JE, Graham DI, Praestgaard AH, Smith DH. A neprilysin polymorphism and amyloid-beta plaques following traumatic brain injury. *J Neurotrauma* 26(8): 1197-1202, 2009. PMID: 19326964. PMCID: PMC2850253

Stein S, Graham DI, Chen X-H, Smith DH. Association between intravascular microthrombosis and cerebral ischemia in traumatic brain injury. *Neurosurgery.* 54(4) 2004. PMID: 15028145

Iwata A, Chen X-H, McIntosh TK, Browne KD, Smith DH: Long-term accumulation of amyloid-ß in axons following brain trauma in rats without persistent upregulation of amyloid precursor protein genes. J Neuropath Exp Neur 61(12): 1056-1068, December 2002. PMID: 12484568

Stein SC, Smith DH: Coagulopathy in traumatic brain injury. Neurocritical Care 1(4): 479-488, 2004. PMID: 16174954

Iwata A, Browne KD, Chen X-H, Yuguchi T, Smith DH: Traumatic brain injury induces biphasic upregulation of ApoE and ApoJ protein in rats. J. Neurosci Res 82(1): 103-114, October 2005. PMID: 16118797

Iwata A, Stys PK, Wolf JA, Chen X-H, Taylor AG, Meaney DF, Smith DH: Traumatic Axonal Injury Induces Proteolytic Cleavage of the Voltage-gated Sodium Channels Modulated by Tetrodotoxin and Protease Inhibitors. J Neurosci 24(19): 4605-4613, May 2004. PMID: 15140932

Iwata A, Browne KD, Chen X-H, Yuguchi T, Smith DH: Traumatic brain injury induces biphasic upregulation of ApoE and ApoJ protein in rats. J. Neurosci Res 82(1): 103-114, October 2005.

Ongoing Research Support:

R01 NS038104-10A1 (PI: D. Smith) 6/1/2010 - 05/31/2015 2 calendar months
NIH NINDS
Pathophysiology of Traumatic Axonal Injury. The goal of this project is to evaluate mechanisms of amyloid-ß accumulation in damaged axons and deposition in the brain following traumatic brain injury. The key aim of this grant is to elucidate the mechanistic link between a history of traumatic brain injury and an increased risk of developing Alzheimer's disease (AD), by identifying shared pathologic pathways. (No cost extension year)

R01 NS048949 (PI: D. Smith) 03/01/2006 – 02/28/2011 (No salary support)

NIH NINDS
Spinal Cord Repair with Nerve Constructs. The key aims are to use engineered nervous tissue constructs to repair spinal cord injury. The efficacy of this treatment will be evaluated in rat spinal cord injury models of lateral hemisection of the thoracic spinal cord and complete transection of the thoracic spinal cord.

T32-NS043126 (PI: D. Smith) 07/01/2008 - 06/30/2013 No salary support
NIH NINDS
Brain Injury Training Grant. The principal goal of the brain injury training grant is to provide an excellent mentoring environment for M.D. and Ph.D. trainees to prepare them for careers in nervous system injury research. Our trainees acquire basic science research skills that address the etiology, pathogenesis, diagnosis, treatment, and prevention of injury to the nervous system, such as traumatic brain injury (TBI) and cerebral ischemia (stroke).

P01-NS056202 (PI: D. Smith) 08/01/2008 - 07/31/2013 4.2 calendar months
NIH
Mild Traumatic Brain Injury and Diffuse Axonal Injury. The overall program project seeks to understand the specific cellular and molecular mechanisms underlying cell death and dysfunction to further the development of therapeutic strategies targeted to treat human brain injury.

P01-NS056202-02S1 (PI: D. Smith) 09/39/2009 - 08/31/2011 (ARRA, No salary support)
NIH
Mild Traumatic Brain Injury and Diffuse Axonal Injury. The overall program project seeks to understand the specific cellular and molecular mechanisms underlying cell death and dysfunction to further the development of therapeutic strategies targeted to treat human brain injury.

P01-NS056202-03S1 (PI: D. Smith) 08/01/2008 - 07/31/2013 No salary support
NIH
Mild Traumatic Brain Injury and Diffuse Axonal Injury. The overall program project seeks to understand the specific cellular and molecular mechanisms underlying cell death and dysfunction to further the development of therapeutic strategies targeted to treat human brain injury.

R01-HD057355 (PI: W. Armstead) 03/20/2008-02/28/2013 1.2 calendar months
NIH/NICHD
Plasminogen activators and NMDA after brain injury. The major goals of the project are to: (1) Characterize the relationship between plasminogen activators and NMDA receptor activation in cerebral hemodynamics following brain injury as a function of age; (2) Investigate the role of MAPK isoforms and LRP as the mechanism by which plasminogen activators and NMDA receptor activation control cerebral hemodynamics following brain injury as a function of age; and (3) Determine the association between plasminogen activators and NMDA receptor induced impairment of cerebral hemodynamics and histopathology following brain injury as a function of age. Role: Co-Investigator

W81XCWH-10-1-0941 (PI: D. Smith) 09/30/2010-09/29/2013 2.4 calendar months
Department of Defense
Spinal Cord Repair with Engineered Nervous Tissue. This project involves a unique neural tissue engineering strategy to create transplantable living nerve constructs for spinal growth repair.

N/A (PI: D. Smith) 04/16/2010-04/15/2011 0.6 calendar months
Acorda Therapeutics
Evaluation of the efficacy of GGF2 treatment in a rat model of traumatic brain injury and in an in vitro model of traumatic brain injury. This study will focus on if and how neuregulins reduce the destructive effects of TBI on the brain.

Completed:

R01 HL077760 (PI: Higazi) 04/01/2006 - 03/31/2010 .72 calendar months
NIH NHLBI
tPA in traumatic brain injury. This project evaluates the protective and deleterious roles of tPA in models of traumatic brain injury. Role: Co-I

R01-NS053410 (PI: W. Armstead) 06/01/2006 - 05/31/2010 1.08 calendar months
NIH
Plasminogen activators and cerebral ischemic injury. The goal of this project is to characterize the relationship between plasminogen activators and cerebral hemodynamics after hypoxia/ ischemia, and investigate the role of MAPK as the mechanism by which plasminogen activators control cerebral hemodynamics post insult; Changes in the MAPK isoform expression profile result in impaired cerebral hemodynamics and neuron cell loss. Also we seek to determine the association between impaired cerebral hemodynamics and histopathology post insult. Role: Co-Investigator

Pending:

1U24NS069540-01A1 (PI: D. Smith) 04/01/2010-03/31/2015 1.2 calendar months
NIH/NINDS
Brain archive for traumatic brain injury.
Traumatic brain injury (TBI) is an extremely common and often devastating health problem. Understanding the pathology of human TBI will be imperative to the development of potential therapeutic interventions. Here we aim to develop the world's most comprehensive, TBI brain tissue archive - an international resource for translational TBI research.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Goshgarian, Harry G.	Professor of Anatomy/Cell Biology
eRA COMMONS USER NAME (credential, e.g., agency login) aa0845	

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.*)

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Massachusetts	B.S.	1971	Zoology
University of Michigan	M.S.	1973	Anatomy
University of Michigan	Ph.D.	1975	Anatomy
Section on Neural Development/Regeneration, Lab of Neurochemistry, National Institutes of Health	Postdoc	1975-1977	Neurochemistry

A. Personal Statement

I have 33 years of experience studying spinal cord injury-induced plasticity and recovery of respiratory muscle function in rats and also have experience in carrying out clinical studies in SCI patients to improve respiratory muscle function after SCI. In addition, I have considerable experience in the pathophysiology of neurotrauma focusing on the effect of drugs on inducing motor recovery. I have expertise in numerous anatomical, physiological, pharmacological and molecular techniques and have a strong knowledge of the literature in neurotrauma. I've been PI on numerous NIH grants including a NIH MERIT Award.

B. Positions and Honors.

Positions and Employment:

08/01/77 – 08/31/82	Assistant Professor of Anatomy, Wayne State University
09/01/81 – 08/31/90	Associate Professor of Anatomy, Wayne State University
10/11/84 – present	Associate in Orthopedic Surgery, Wayne State University
09/01/90 – present	Professor of Anatomy/Cell Biology, Wayne State University
06/01/2000 – present	Associate in Internal Medicine, Wayne State University

Honors:

NIH MERIT AWARD (NICHD), 1999-2009
Excellence Award, Academy of Spinal Cord Injury Professionals (APS Section), 2010.

C. Selected peer-reviewed publications (from a PubMed list of 85 publications):

1. Hadley, S.D., Walker, P.D., and **Goshgarian, H.G.** Effects of the serotonin synthesis inhibitor p-CPA on the expression of the crossed phrenic phenomenon 4 h following C2 spinal cord hemisection. *Exp. Neurol.* 160(2): 479-488, 1999. PMID: 10619565 doi:10.1006/exnr.1999.7240
2. Zhou, S.Y. and **Goshgarian, H.G.** Effects of serotonin on crossed phrenic nerve activity in cervical spinal cord hemisected rats. *Exp. Neurol.* 160(2): 446-453, 1999. PMID: 10619561 doi:10.1006/exnr.1999.7213
3. Zhou, S.Y. and **Goshgarian, H.G.** 5-Hydroxytryptophan-induced respiratory recovery after cervical spinal cord hemisection in rats. *J. Appl. Physiol.* 89(4): 1528-1536, 2000. PMID: 11007592 http://jap.physiology.org/cgi/reprint/89/4/1528
4. Zhou, S.Y., Basura, G.J., and **Goshgarian, H.G.** Serotonin(2) receptors mediate respiratory recovery after cervical spinal cord hemisection in adult rats. *J. Appl. Physiol.* 91(6): 2665-2673, 2001. PMID: 11717232 http://jap.physiology.org/cgi/reprint/91/6/2665

5. Zhou, S.Y., Castro-Moure, F., and **Goshgarian, H.G**. Activation of a latent respiratory motor pathway by stimulation of neurons in the medullary chemoreceptor area of the rat. *Exp. Neurol.* 171(1): 176-184, 2001. PMID: 11520132 doi:10.1006/exnr.2001.7740
6. Basura, G.J., Zhou, S.Y., Walker, P.D., and **Goshgarian, H.G**. Distribution of serotonin 2A and 2C receptor mRNA expression in the cervical ventral horn and phrenic motoneurons following spinal cord hemisection. *Exp Neurol.* 169(2): 255-263, 2001. PMID: 11358440 doi:10.1006/exnr.2001.7682
7. Phillis, J.W. and **Goshgarian, H.G**. Adenosine and neurotrauma: therapeutic perspectives. *Neurol. Res.* 23(2-3): 183-189, 2001. PMID: 11320597
8. Nantwi, K.D. and **Goshgarian, H.G**. Alkylxanthine-induced recovery of respiratory function following cervical spinal cord injury in adult rats. *Exp. Neurol.* 168(1):123-134, 2001. PMID: 11170727 doi:10.1006/exnr.2000.7581
9. Basura, G.J., Nantwi, K.D., and **Goshgarian, H.G**. Theophylline-induced respiratory recovery following cervical spinal cord hemisection is augmented by serotonin 2 receptor stimulation. *Brain Res.* 956(1): 1-13, 2002. PMID: 12426040 http://www3.interscience.wiley.com/cgi-bin/fulltext/118951700/PDFSTART
10. Nantwi, K.D. and **Goshgarian, H.G**. Actions of specific adenosine receptor A1 and A2 agonists and antagonists in recovery of phrenic motor output following upper cervical spinal cord injury in adult rats. *Clin. Exp. Pharmacol. Physiol.* 29(10): 915-923, 2002. PMID: 12207572 http://www3.interscience.wiley.com/cgi-bin/fulltext/118951700/PDFSTART
11. Nantwi, K.D., Basura, G.J., and **Goshgarian, H.G**. Adenosine A1 receptor mRNA expression and the effects of systemic theophylline administration on respiratory function 4 months after C2 hemisection. *J. Spinal Cord Med.* 26(4): 364-371, 2003. PMID: 14992338
12. Nantwi, K.D., Basura, G.J., and **Goshgarian, H.G**. Effects of long-term theophylline exposure on recovery of respiratory function and expression of adenosine A1 mRNA in cervical spinal cord hemisected adult rats. *Exp. Neurol.* 182(1): 232-239, 2003. PMID: 12821393 doi:10.1016/S0014-4886(03)00109-2
13. **Goshgarian, H.G.** The crossed phrenic phenomenon: a model for plasticity in the respiratory pathways following spinal cord injury. *J. Appl. Physiol.* 94(2): 795-810, 2003. PMID: 12531916 http://jap.physiology.org/cgi/reprint/94/2/795
14. Zimmer, M.B. and **Goshgarian, H.G**. Spontaneous crossed phrenic activity in the neonatal respiratory network. *Exp. Neurol.* 194(2): 530-540, 2005. doi:10.1016/j.expneurol.2005. PMID: 16022876
15. Nantwi, K.D. and **Goshgarian, H.G**. Adenosinergic mechanisms underlying recovery of diaphragm motor function following upper cervical spinal cord injury: potential therapeutic implications. *Neurol. Res.* 27(2): 195-205, 2005. PMID: 15829183
16. Bascom, A.T., Lattin, C.D., Aboussouan, L.S., and **Goshgarian, H.G**. Effect of acute aminophylline administration on diaphragm function in high cervical tetraplegia: a case report. *Chest* 127(2): 658-661, 2005. PMID: 15706011. http://www.chestjournal.org/cgi/reprint/127/2/658
17. Bae, H., Nantwi, K.D., and **Goshgarian, H.G**. Recovery of respiratory function following C2 hemi and carotid body denervation in adult rats: influence of peripheral adenosine receptors. *Exp. Neurol.* 191(1): 94-103, 2005. PMID: 15589516 doi:10.1016/j.expneurol.2004
18. Tzelepis, G.E., Bascom, A.T., Badr, S.M., and **Goshgarian, H.G**. Effects of theophylline on pulmonary function in patients with traumatic tetraplegia. *J. Spinal Cord Med.* 29(3): 227-233, 2006. PMID: 16859226 http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=1864809&blobtype=pdf
19. Zimmer, M.B. and **Goshgarian, H.G**. Spinal activation of serotonin 1A receptors enhances latent respiratory activity after spinal cord injury. *J. Spinal Cord Med.* 29(2):147-155, 2006. PMID: 16739558 http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=1864797&blobtype=pdf
20. Minor, K.H., Akison, L.K., **Goshgarian, H.G.**, and Seeds, N.W. Spinal cord injury-induced plasticity in the mouse--the crossed phrenic phenomenon. *Exp. Neurol.* 200(2): 486-495, 2006. PMID: 16631169 doi:10.1016/j.expneurol.2006
21. Alilain, W.J. and **Goshgarian, H.G.** MK-801 upregulates NR2A protein levels and induces functional recovery of the ipsilateral hemidiaphragm following acute C2 hemisection in adult rats. *J. Spinal Cord Med.* 30(4): 346-354, 2007. PMID 17853656. http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=2031932&blobtype=pdf

22. Zimmer, M.B., Nantwi, K., and **Goshgarian, H.G**. Effect of spinal cord injury on the respiratory system: basic research and current clinical treatment options. *J. Spinal Cord Med.* 30(4): 319-330, 2007. PMID 17853653. http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=2031930&blobtype=pdf
23. Zimmer, M.B. and **Goshgarian H.G.** Spinal cord injury in neonates alters respiratory motor output via supraspinal mechanisms. *Exp. Neurol.* 206(1):137-145, 2007. PMID: 17559837. doi:10.1016/j.expneurol.2007
24. Zimmer, M.B. and **Goshgarian, H.G**. GABA, not glycine, mediates inhibition of latent respiratory motor pathways after spinal cord injury. *Exp. Neurol.* 203(2): 493-501, 2007. PMID: 17046753. http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=1852446&blobtype=pdf
25. Zimmer, M.B., Nantwi, K., and **Goshgarian, H.G**. Effect of spinal cord injury on the neural regulation of respiratory function. *Exp. Neurol.* 209(2): 399-406, 2008. PMID: 17559837. doi:10.1016/j.expneurol.2007.
26. Kajana, S. and **Goshgarian, H.G**. Administration of phosphodiesterase inhibitors and an adenosine A1 receptor antagonist induces phrenic nerve recovery in high cervical spinal cord injured rats. *Exp. Neurol.* 210(2): 671-680, 2008. PMID: 18289533 doi:10.1016/j.expneurol.2007.12.021 .
27. Alilain, W.J, and **Goshgarian, H.G.** Glutamate receptor plasticity and activity-regulated cytoskeletal associated protein regulation in the phrenic motor nucleus may mediate spontaneous recovery of the hemidiaphragm following chronic cervical spinal cord injury. *Exp Neurol.* 212(2): 348-357, 2008. PMID: 18534577 doi:10.1016/j.expneurol.2008.
28. Kajana, S. and **Goshgarian, H.G**. Spinal activation of the cAMP-PKA pathway induces respiratory motor recovery following high cervical spinal cord injury. *Brain Res.* 1232:206-13, 2008. PMID: 18656458. doi:10.1016/j.brainres.2008.07
29. Huang, Y. and **Goshgarian, H.G.** Identification of the neural pathway underlying spontaneous crossed phrenic activity in neonatal rats. *Neuroscience* 163(4):1109-18. PMID: 19596054.
30. Kajana, S. and **Goshgarian, H.G.** Systemic administration of rolipram increases medullary and spinal cAMP and activates a latent respiratory motor pathway after high cervical spinal cord injury. *J Spinal Cord Med.* 32(2):175-82, 2009. PMID: 19569465.
31. Huang, Y. and **Goshgarian, H.G.** The potential role of phrenic nucleus glutamate receptor subunits in mediating spontaneous crossed phrenic activity in neonatal rat. *Int J Dev Neurosci.* (5):477-83, 2009. PMID: 19446017.
32. Huang, Y. and **Goshgarian, H.G.** Postnatal conversion of cross phrenic activity from an active to latent state. *Exp Neurol.* 219(1):66-73, 2009. PMID: 19416665.

D. Research Support.

Ongoing Research Support:

R37 HD31550 Goshgarian (PI) 07/04/04-05/31/11
NIH/NICHD
Functional Plasticity in the Mammalian Spinal Cord
The above is presently in a no-cost extension of a 10 year NIH MERIT Award whose goals are to understand the underlying mechanisms related to recovery of respiratory muscles paralyzed by cervical spinal cord injury.
Role: PI

No. 160310 Goshgarian (PI) 07/1/10-06/30/12
Craig H. Neilsen Foundation
Molecular Basis for Drug-Induced Recovery of Breathing after Spinal Cord Injury.
The goal of this grant is to delineate the intracellular signaling pathways and molecular mechanisms for both short-term and long-term recovery induced by phosphodiesterase inhibitors in C2 spinal cord hemisected rats.
Role: PI

Completed Research Support:

No. 69601 Goshgarian (PI) 01/1/08-12/31/08
Craig H. Neilsen Foundation (Program: Program/Opportunity Grants)
2008 American Paraplegia Society (APS) Annual Conference
The goal of this grant is to help sponsor the 2008 APS Annual Conference. This is a meeting of over

500 health care professionals dedicated to the care of spinal cord injury patients. Advances in research and patient care are discussed at this meeting. Dr. Goshgarian is the Chairman of the APS Program Committee which has the responsibility of developing the entire program for this meeting. This relationship with the APS has existed since 1989 and will facilitate technological translation to the clinic after the aims of the current application are completed.
Role: PI

RO1 HD 35766 Nantwi (PI) 09/1/2002-08/31/06
NIH/NICHD
Drug-induced Motor Recovery after Spinal Cord Injury.
The goal of this grant is to identify adenosinergic mechanisms for drug-induced motor recovery in the diaphragm after C2 spinal cord injury.
Role: Co-investigator

No. (Number unknown) Goshgarian (PI) 01/1/07-12/31/07
Craig H. Neilsen Foundation (Program: Program/Opportunity Grants),
2007 American Paraplegia Society Annual Conference
The goal of this grant is to help sponsor the 2007 APS Conference. The purpose of the grant is the same as the 2008 Neilsen grant listed above.
Role: PI

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
William Andrew Kofke, M.D., M.B.A. FCCM	Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Bucknell University, Lewisburg, PA	BS	1974	Chemistry
University of Pittsburgh, Pittsburgh, PA	MD	1978	Medicine
Mercy Hospital, Pittsburgh, PA		1978-1979	Internship
Massachusetts General Hospital, Boston, MA		1979-1981	Anesthesiology
Massachusetts General Hospital, Boston, MA		1982-1983	Critical Care
University of Pittsburgh, Pittsburgh, PA		1992-1993	Neuroscience Sabbatical
West Virginia University, Morgantown, WV	MBA	1999	Business Admin.

Personal Statement

I am the founder and codirector of the UPenn NeuroICU. I have a long standing interest in prevention of secondary brain injury after a variety of primary neurologic insults. Too many patients are admitted to the neuroICU and, despite our best efforts, go on to develop new brain damage… essentially nosocomial stroke. I am pleased to be asked to serve on the conflict resolution committee should any disparity occur between the work of Drs. Kreipke and Armstead. As such I am hoping to contribute to development of more knowledge that may help decrease secondary brain injury after TBI.

A. Positions and Honors.

Clinical and Academic Positions

1978-1979 Flexible Intern, Mercy Hospital, Pittsburgh, PA
1979-1981 Resident in Anesthesia, Harvard, Mass. Gen. Hospital (MGH)
1981-1982 Instructor in Anesthesia, Harvard, MGH
1982-1983 Clinical Fellow in Anesthesia (Critical Care and Neuroanesthesia), Harvard, MGH
1983-1988 Assistant Professor, Pennsylvania State University
1988-1995 Assist/Assoc Prof, Dir. Neuroanesthesia, Depts. of Anes/CCM & Neurosurg, Univ. of Pittsburgh
1991-1992 Sabbatical; Faculty of Arts & Sciences, Department of Behavioral Neuroscience, Center for Neuroscience, University of Pittsburgh (Graduate neuroscience courses)
1996-1997 Professor, Anesthesia/CCM and Neurosurgery, University of Pittsburgh
1997-2001 Professor, Anesth and Neurosurg, Dir Neuroanesthesia, Vice Chair, West Virginia University
2001- Professor, Anesthesia and Neurosurgery, Director Neuroanesthesia, University of Pennsylvania
2005- Co-Director Neurocritical Care, University of Pennsylvania

Honors and Certifications

1970 Gulf Oil Corporation Scholarship
1973 Omicron Delta Kappa
1976 Excellence in Research Award, AMSA-UTMB
1978 Kinderer Award in Otolaryngology, Univ. of Pittsburgh
1983 Diplomate, American Board of Anesthesiology
1985 Parker B. Francis Investigator in Anesthesiology
1986 Certification Crit Care Med. Am. B. Anesthesiology
1990 Association of University Anesthesiologists (Elected)
1991 Fellow, American College of Critical Care Medicine (Elected)
1992 Continued Demonstration of Qualifications, ABA
2007 Certification Neurocritical Care
1995 Pennsylvania Soc. Of Anesthesiologists Resident Research Competition, 1st first place Elizabeth Sinz, Research Trainee (Co-investigator)
1995 Am. Soc. of Anesthesiologists Resident Research Competition, 2nd place, Elizabeth Sinz
1995 Soc. of Neurosurg. Anesth Crit Care Young Investigator Award Elizabeth Sinz, research trainee

B. **Selected Refereed Publications (82 total)**

1. Kofke WA, Nemoto EM, Hossman K-A, Taylor F, Kessler PD, Stezoski SW: Brain blood flow and metabolism after global brain, ischemia and post-insult thiopental therapy in monkeys. Stroke 10:554,1979 http://stroke.ahajournals.org/cgi/reprint/10/5/554
2. Kofke WA et al: Isoflurane for refractory status epilepticus: A clinical series. Anesthesiology 71:653, 1989. http://journals.lww.com/anesthesiology/Abstract/1989/11000/Isoflurane_for_Refractory_Status_Epilepticus_A.5.aspx
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Selected Books and Chapters (54 total)

1. Kofke WA, Levy JH (Eds), Postoperative Critical Care Procedures of the MGH. Little, Brown, 1986
2. Kofke WA. Perioperative management of acute CNS injury. In Perioperative Medicine Fleisher L et al (eds), 2007 .

3. Kofke WA. Protection of the Central Nervous System in Surgical Patients in Principles of Anesthesiology Longnecker DE et al (eds) 2007.
4. Kofke WA. Future Advances in Neuroanesthesia. in Anesthesia and Neurosurgery, Cottrell JE and Young WL (eds) Elsevier, 2009.
5. Kofke WA, Brown RJ; Postoperative Care in Bhardwaj A and Mirski MA (eds) Handbook of Neurocritical Care, 2nd edition Humana, in press, 2010.
6. Kofke WA: Critical Neuropathophysiology, in Fink M, etal (eds) Textbook of Critical Care, 6th Ed, 2010 in press.

Other Support

ACTIVE

1R21NS061857-01A2 (Kofke) 04/01/2010 – 03/31/2012 0.89 Calendar
NIH/NINDS $137,500
Validation of NIRS CBF with XeCTCBF

The major goals of this project are to validate a new noninvasive monitor of regional cerebral blood flow with XeCTCBF in subarachnoid hemorrhage patients.

(Kofke) 10/01/10-09/30/11 0 Calendar
Covidien $16,900
The Use of the Bispectral Index Monitoring System to Detect Seizures in a Brain-Injured Population: A Prospectively Collected Database

The major goal of this project is to evaluate BIS monitoring as a screening tool for seizures in NeuroICU patients

(Kofke) 07/01/10-06/30/11 0.12 Calendar
Somanetics (now Covidien) $12,302
Enhanced Cerebral Oximetry During Intracranial Angiography

The major goal of this project is to test a new infrared spectroscopy patch in neuroradiology patients

PENDING

1 R21 NS061074-01A2 (Kofke) 12/1/10-11/30/12 1.2 Calendar
NIH/NINDS $137,500
Genes in Human Brain Ischemia

The goal of this project is to ascertain genes that contribute to susceptibility to brain damage in humans after deep hypothermic circulatory arrest. This grant is deemed highly likely to receive funding but an award decision by council is pending.

Program Director/Principal Investigator (Last, First, Middle): Kreipke, Christian W.

RESOURCES

Follow the 398 application instructions in Part I, 2.7 Resources.

Year 1 Rat TBI studies (Wayne State University)

Office Space

Dr. Kreipke has a 300 sq ft office which is entirely dedicated to him. He has 700 sq ft additional space for technicians, postdoctoral fellows, and graduate students. His office contains one computer while the additional office space houses two desktops and two laptops. Each Co-I has his/her own office with computer.

Animal Housing and Care

Facilities for the care and housing of experimental animals are available in the basement of Scott Hall. These resources are operated by the University Department of Laboratory Animal Resources. At WSU, all animals used for biomedical research at the medical center are housed in modern animal care facilities with excellent supervisory and veterinary support.

Surgery

Dr. Kreipke currently manages a 700 sq. ft. laboratory (9320 Scott Hall) fully IACUC/DLAR approved for rodent surgery. All necessary tools for surgery are currently available to his laboratory. A modest request for maintaining such supplies (sutures, scalpels, gauze, cotton, replacement hair clipper blades, etc.) is included in the budget. In addition, Dr. Kreipke also has a 400 sq ft. facility for post-operative care, housing and maintenance which is also fully IACUC/DLAR approved.

(Resources continued on the following continuation pages)

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

CBF determination

Wayne State University, in association with Harper Hospital, houses a MRI facility core which contains the 4.7 T Brucker magnet fully equipped which arterial spin labeling software which will be used for all determination of CBF. While this is a shared magnet, the addition of a new 7.0 T magnet has greatly decreased the need for usage of the 4.7 T, allowing full access for Dr. Kreipke and his team.

Histological Core

Dr. Kreipke's laboratory is equipped with two cryostats for tissue cutting and a dedicated room to histological analyses. Furthermore, Dr. Kreipke possesses a state-of-the-art Nikkon microscope fitted with camera and appropriate software for detecting and quantifying both light field and fluorescence data.

Behavioral suite

Dr. Kreipke manages a 400 sq ft. (9332 Scott Hall) behavioral core which presently includes two radial arm mazes which are fully automated using Smart™ Version 2.5 software (San Diego Instruments, San Diego, CA). A modest request for two additional radial arm mazes is included in the budget.

Years 2 and 3 Porcine TBI studies (University of Pennsylvania)

Office Space

Dr. Armstead has a 100 sq ft office dedicated entirely to him with a desktop computer. His office is connected to his laboratory. Co-Is each have their own office with computers available to them.

Animal Housing and Care

Pigs are purchased from a commercial breeder and are maintained in a University animal facility. A dedicated sterile surgical suite will be available for survival traumatic brain injury studies of pigs. These resources are operated by the University Department of Laboratory Animal Resources.

Surgery

Dr. Armstead has a 380 sq ft laboratory and currently possesses a lateral fluid percussion brain injury device (designed and built by Medical College of Virginia), small animal ventilators, Siemens 248 Blood Gas analyzer, balance, pumps, and surgical supplies. A modest request for a dedicated lateral fluid percussion brain injury device is included in the budget since it would be cumbersome and difficult to move on a continual rotating basis the current device used for acute studies between the Armstead lab and a sterile surgical suite for survival studies.

CBF determination

Program Director/Principal Investigator (Last, First, Middle): Kreipke, Christian W.

CHECKLIST

TYPE OF APPLICATION *(Check all that apply.)*

☐ NEW application. *(This application is being submitted to the PHS for the first time.)*

☒ RESUBMISSION of application number: _____
(This application replaces a prior unfunded version of a new, renewal, or revision application.)

☐ RENEWAL of grant number: _____
(This application is to extend a funded grant beyond its current project period.)

☐ REVISION to grant number: _____
(This application is for additional funds to supplement a currently funded grant.)

☐ CHANGE of program director/principal investigator.
Name of former program director/principal investigator: _____

☐ CHANGE of Grantee Institution. Name of former institution: _____

☐ FOREIGN application ☐ Domestic Grant with foreign involvement List Country(ies) Involved: _____

INVENTIONS AND PATENTS *(Renewal appl. only)* ☐ No ☐ Yes
If "Yes," ☐ Previously reported ☐ Not previously reported

1. PROGRAM INCOME ***(See instructions.)***
All applications must indicate whether program income is anticipated during the period(s) for which grant support is request. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)
	$0.00	

2. ASSURANCES/CERTIFICATIONS ***(See instructions.)***
In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable. Descriptions of individual assurances/certifications are provided in Part III and listed in Part I, 4.1 under Item 14. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

3. FACILITIES AND ADMINSTRATIVE COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

☒ DHHS Agreement dated: 04/29/2010 ☐ No Facilities And Administrative Costs Requested.

☐ DHHS Agreement being negotiated with _____ Regional Office.

☐ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION* *(The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)*

a. Initial budget period:	Amount of base $	828,434	x Rate applied	52.00	% = F&A costs	$	430,786
b. 02 year	Amount of base $	41,168	x Rate applied	52.00	% = F&A costs	$	21,407
c. 03 year	Amount of base $	42,403	x Rate applied	52.00	% = F&A costs	$	22,050
d. 04 year	Amount of base $	98,321	x Rate applied	52.00	% = F&A costs	$	51,127
e. 05 year	Amount of base $	0	x Rate applied	0.00	% = F&A costs	$	0
					TOTAL F&A Costs	$	525,370

*Check appropriate box(es):
☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*
☐ Off-site, other special rate, or more than one rate involved *(Explain)*
Explanation *(Attach separate sheet, if necessary.)*:

4. DISCLOSURE PERMISSION STATEMENT: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)? ☐ Yes ☐ No

We thank the review panel for their thorough examination of this proposal and for their insight into making this proposal stronger. We appreciate the overall enthusiasm for pursuing Clazosentan as a potential clinically relevant treatment for TBI. We have carefully considered each critique and have incorporated the review panel's ideas into this revised proposal. The following outlines the major changes in this revision and is organized by category:

Overall Impact: The main issues expressed were: 1) the choice of models used in this proposal are diffuse and may not represent the human condition which includes both diffuse and focal injury, 2) how the induced vasodilation will affect bleeding, 3) whether the induced levels of hypoperfusion are linked to dysfunction and whether ET-1 is the best target, 4) the research design is limited with respect to efficacy window, rationale for further rat studies, and outcome measures (i.e., a lack of histological analysis) and 5) the lack of proper rationale for the team at Wayne State University, including the PI, Dr. Kreipke. As to the choice of models, we have carefully selected these models which, though we recognize that both weight-drop and fluid percussion models have been labeled as "diffuse" injury models, have shown to include a mixture of diffuse and focal injury to mimic most closely human closed head injury (Gennarelli J Neurotrauma 66:409, 1994). With respect to the issue of bleeding as being a confound, we would like to point out, first, that Clazosentan is currently undergoing clinical trial for subarachnoid hemorrhage with positive results and, thus, is likely ideally suited to address microhemorrhage and, second, that as we have both published and included as preliminary data, Clazosentan, at the doses we have suggested, seems to stabilize vasoconstriction more than induce vasodilation and, thus, we do not see this as a major confound. Since we do not observe bleeding after TBI, our model is distinct from SAH. We would also like to point out that, as reviewed in Bramlett and Dietrich (2004), the hypoperfusion seen in both animal models and human TBI, while not being the same levels as classically seen as ischemic, when combined with diffuse axonal injury, edema, and other pathologies contributes to overall neuronal injury and, thus, is likely linked to overall dysfunction. ET-1, while being only one pathway to vasoconstriction, has shown to be an important target in that both of our laboratories have explored other vasoactive substances (e.g., vasopressin and thromboxane) and shown that none have the magnitude of response as that seen with the ET-1 system. We completely agree that our initial inclusion of only two timepoints was limited. Therefore, in consultation with program officials, have included a 4 hour and a 12 hour timepoint to expand the efficacy window. Further, in doing so, we solidify the rationale for conducting such comprehensive studies in rat to inform our pig studies in Year 2 and 3 (i.e., rat studies provide a cheaper less sentient animal for high throughput analysis to inform selection of timepoints for more translational pig studies). Finally, we offer that any confusion over the selection of the team at Wayne State University, including the PI, was our lack of proper rationale for each investigator. We have expanded the research team, feel that the better explanation of how each member will contribute and the fact the several key studies have been already conducted at Wayne will help clarify our roles and will restore confidence in our team.

Significance: all concerns were addressed in overall impact

Investigators: While reviewers 1 and 2 felt that this was a major strength, reviewer 3 was concerned about the team at Wayne State. We expanded the research team and feel that the inclusion of a stronger rationale for each investigator at Wayne solidifies enthusiasm for our team.

Innovation: This was generally thought to be a strength. However one concern was raised with regards as to how this proposal is distinct from others that have targeted hypoperfusion. We agree that targeting hypoperfusion is not innovative per se. However, this proposal is the first to use a highly specific ETrA antagonist to address the milestone that it is the brain milieu after TBI in the setting of hypoperfusion – and endothelin release in particular – that drives outcome post injury.

Approach: First, it was suggested that we include other areas of CBF analysis. Since we always conduct whole brain MRI scans, we can analyze various areas. In fact, we have included cortical analyses in our revised preliminary data. Second, it was unclear how a single bolus would exert long-term effects. Even though our preliminary results suggest that Clazosentan does exert effects to ameliorate hypoperfusion up to 48 hours, we are conducting on-going mechanistic studies in the laboratory to understand how ETrA antagonists exert long-term effects, however these studies are beyond the scope of this proposal which does not allow for mechanistic studies. Third, it was suggested that we reevaluate the pig behavioral data to include a more comprehensive analysis of individual measures. We agree and have reworked our analysis. Other areas where we have improved this proposal is that we have included better statistical/power analyses, we have made our go-no-go statements more clear, and have included histological analyses to both address the issue of cell death/neuroprotection and to deal with issue of spatial alterations. While it was suggested that we measure ICP and edema and may want to include a range of injury severity, we would like to point out that we have recently published such data and feel that the additional animals required for such analyses would go beyond the scope of this mechanism.

Environment: addressed in overall impact.

A. Specific AIMS

Traumatic brain injury (TBI), the leading cause of death and disability amongst our youth (CDC, 2004) and the signature injury in the "War on Terrorism", is characterized by three major pathologies: 1) cerebral edema, 2) diffuse axonal injury (DAI), and 3) enhanced vasoreactivity leading to hypoperfusion of the brain's parenchyma. To date, over 25 clinical trials have been developed to improve outcome by addressing the first two pathologies. None have been successful (Povlishock, 2008). However, to our knowledge no one has developed studies to address the clinical relevance of hypoperfusion.

Using a model of mixed focal/disuse brain injury, Dr. Armstead published that endothelin-1 (ET-1), a powerful vasoconstrictor, plays a critical role in mediating vasoconstriction following TBI (Armstead, 1996). This work led to multiple studies that focused on the role of ET-1 and its receptors, ETrA (primarily mediates vasoconstriction) and B (primarily mediates vasodilation), in mitigating hypoperfusion following TBI (Sato and Noble, 1998; Zhang et al., 2000; Armstead, 2004; Kreipke et al., 2009). Several laboratories have published that blocking ETrA using BQ-123, a specific ETrA antagonist, improves cerebral blood flow (CBF) after TBI (reviewed in Armstead, 2004; Kreipke et al., 2009). Dr. Kreipke further studied the effects of hypoperfusion on cellular and behavioral outcome following TBI and showed that BQ-123 reduces the extent of hypoperfusion which, in turn, improves both cellular and behavioral outcome following TBI (Kreipke et al., 2009). While these results show promise, enthusiasm for clinical application of this work has been dampened by a lack of a clinically relevant drug that is specific to ETrA (e.g., Bosentan, a mixed ETrA/B antagonist, causes systemic hypotension and, thus, is not a suitable candidate for improving outcome following TBI). However, in 2007 Actelion developed Clazosentan, which, is the most highly specific ETrA antagonist currently available (10-100X more selective than previously available drugs [Bosentan, Enrasentan, Tezosentan, Darusentan]) (Baltistini et al., 2004) and which is currently undergoing Phase III clinical trial for use after aneurysmal subarachnoid hemorrhage (SAH). While much pertinent information regarding toxicity, absorption, metabolism, etc. has already been gained, to date no one has tested the efficacy of Clazosentan following TBI. To this end, we have begun to conduct preliminary studies which show that Clazosentan recapitulates data gained from BQ-123 (e.g., a 1mg/kg dose administered at 2 h post TBI completely ameliorates hypoperfusion). Clazosentan has the clinical use advantage of possessing an activity profile utilizing intravenous dosing administration devoid of significant systemic peripheral vascular action. Since much of the Investigation of New Drug (IND) enabling studies have already been performed by Actelion in support of the SAH trial and will be shared with us by the company, our proposal will only consider dosing optimization relative to time of administration and time window of therapeutic outcome efficacy.

The **Goal** of this U01 proposal is to test whether **Clazosentan is effective in ameliorating hypoperfusion and, ultimately, improving behavioral outcome following TBI.** In order to accomplish this goal, Drs. Kreipke's and Armstead's teams will combine resources to investigate Clazosentan in two different models of diffuse brain injury, the rodent weight acceleration impact and porcine fluid percussion models. We have, in consultation with Program at NINDS, streamlined this application to focus on blood flow and behavioral outcome. In direct response to reviewer's comment, in this resubmission we will also include histopathological analysis as an additional outcome measure to confirm the potential neuroprotective properties of ETrA antagonism. Therefore, as outcome measures, we will measure, CBF using MRI imaging, *extent of cell injury using standard histological preparations*, and behavioral outcome. Use of two different species and types of TBI that encompass both focal and diffuse injury will strengthen and support the broader applicability of the results to the human. The proposal paradigm will use the rat as a high throughput vehicle for establishing initial efficacy of drug, while the pig will be used to establish translational relevance. The target population in human TBI that will be modeled by our preclinical studies is one of moderate to severe injury that leads to spasm. The following Specific Aims have been developed:

Specific AIMS

1). Test whether a certain dose of Clazosentan, given as either single or multiple bolus injections at a *range of timepoints* will be effective in reducing the extent and duration of hypoperfusion and improve behavioral outcome in a rodent model of TBI.

2). Test whether a certain dose of Clazosentan, given as either single or multiple bolus injections at either *an early or later timepoint post TBI* (times to be determined in Year 1 studies) will be effective in reducing the extent and duration of hypoperfusion and improve behavioral outcome in a porcine model of TBI.

B. Background: Significance and Innovation

Traumatic Brain Injury (TBI) and Brain Pathology: Why focus on blood flow following injury?

TBI results in several major histopathologic events, including among others: cerebral edema which leads to a critical rise in intracranial pressure, diffuse axonal injury which brings about disruption of neural circuits underlying cognitive and motoric behaviors, and alterations in the brain's microcirculation that cause a persistent state of hypoperfusion and improper delivery of vital metabolites to neural tissue. In closed head TBI incidents in humans, all these events are thought to significantly contribute to the ensuing morbidity and mortality encountered in clinical settings. Over 25 clinical trials have been developed aimed at reducing or treating the first two pathologies. None have proven successful in moving forward on a treatment paradigm. However, no one has initiated a clinical trial aimed at alleviating hypoperfusion following TBI. As reviewed in 2001, trauma-induced decreases in cerebral blood flow directly contribute to poor outcome in patients and that understanding management of CBF could be critical to improved outcome (Zwienenberg and Muizelaar, 2001). *Furthermore, as reviewed by Bramlett and Dietrich (2004), the hypoperfusion seen in both animal models and human TBI, while not being the same levels as classically seen as ischemic, when combined with diffuse axonal injury, edema, and other pathologies contributes to overall neuronal injury and, thus, is likely linked to overall dysfunction.* Therefore, our combined laboratories have dedicated the last decade to understanding changes in brain microcirculation following trauma. Both laboratories, using different models of TBI and different species (rat and porcine) have shown that TBI results in a significant reduction of CBF (Armstead 1996; Rafols et al., 2007). This coincides temporally with enhanced vascular stress response (as determined by HSP-70 immunocytochemistry), neuronal injury (Rafols et al., 2007), impairment of ATP-sensitive K+ channels (Armstead, 1999), suppressed celiular energy levels in neurons (Huttemann et al., 2007), and poor cognitive outcome (Kreipke et al., 2007). Taken together, these results suggest that hypoperfusion has a profound effect on outcome after TBI. Therefore, this proposal will seek to reduce the extent and duration of hypoperfusion in order to improve behavioral outcome following TBI.

Endothelin-1 (ET-1), its receptors, and control of CBF following TBI.

Endothelin-1 (ET-1) is a powerful vasoconstrictor which has been localized to brain endothelial cells, glia, and neurons (Rafols, 2007). Acting through its receptors, A (ETrA) and B (ETrB), ET-1 signals downstream molecular changes in smooth muscle (SM) of reacting microvessels, ultimately affecting SM's contractility and the cerebral microcirculation. ET-1, as well as a host of other vascular agents such as adenosine, prostaglandins and nitric oxide (NO) are thought to interact metabolically to modulate vasoconstriction and vasodilation, ultimately maintaining vascular tone in CBF autoregulation (Andersson, 2001). Metabolic disruption of the balance between these substances can lead to altered vascular tone and autoregulatory capacity, such as those seen after TBI (Rafols, 2007).

In peripheral vascular beds there is consensus in the literature regarding the role of ETrA in mediating vasoconstriction (reviewed in Jacobs et al. 2006). We have previously shown that brain ETrA is upregulated following TBI (Rafols, 2007). Attenuation of ETrA in brain resulted generally in an increase in the cross-sectional diameter of cerebral arteries, especially following subarachnoid hemorrhage (Ishikawa et al, 1992). Furthermore, in vivo blockade of ETrA with BQ-123 (a selective ETrA antagonist) caused a reduction of a focal ischemic lesion (Barone et al, 2000).

While ETrB has been also shown to be upregulated in brain following TBI (Rafols, 2007), its role in the control of cerebral microvascular tone is controversial. As such, in peripheral vascular beds, ETrB has been shown to mediate both vasoconstriction and vasodilation. In one study, for example, elevation of blood pressure induced by ET-1 application was eliminated by BQ-788, a selective ETrB anatagonist (Ishikawa et al., 1994). Several other studies, however, have shown that ETrB antagonism has no effect on blood pressure (reviewed in Pollack and Schneider, 2006). One study, in particular, showed that, in pial vessels, BQ-788 abolished BQ-3020 (a selective ETrB agonist)-induced vasodilation (Touzani et al., 1997).

Collectively, results from our laboratories suggest that altered ETrA expression may underlie dysfunctional control of microcirculation after TBI. Thus by blocking ETrA we were able to restore CBF to normal levels after trauma using BQ-123 (Kreipke et a., 2009). In contrast ETrB blockade did not ameliorate hypoperfusion after trauma (Kreipke et al., 2009). In fact, at 48 hours post injury, 20nmol BQ-788 further

decreased CBF suggesting that it has a deleterious role in the control of vascular tone after injury. By blocking ETrB, more ET-1 may be available for ETrA, thus enhancing vasoconstriction. Similarly, ETrB may participate in mediating the hyperemia detected with ETrA antagonism. It is possible that ETrA antagonism preferentially shifts ET-1 binding to the ETrB receptor which could yield increased vasodilation and, hence, hyperemia (Begnini, 2007). Taken together, these results suggest that, while the ET-1 system may not be the only cause of dysregulated microvascular tone, pharmacological strategies aimed at selectively blocking ETrA with limited to no blockade of ETrB could show promise in improving outcome following TBI.

Innovation: Why use Clazosentan?

Brief history of development

In 1995 Luscher and Wenzel published one of the first reviews which characterized ET-antagonists as potential clinical therapeutics for vascular disorders (Luscher and Wenzel, 1995). In 1999, Benigni and Remuzzi published a follow-up which summarized data from pre-clinical and clinical studies which showed promise for specific ETrA antagonists in controlling hypertension. Bosentan, a mixed antagonist (ETrA and B) was discussed and clinical trial suggested that the potential opposing effects of ETrA and B may render Bosentan less effective (Benigni and Remuzzi, 1999). In 2003, it was reported that, after thorough investigation of ongoing clinical trial, Bosentan had some success in control of pulmonary arterial hypertension, however was not more effective than other, non-endothelial specific drugs (Krum and Liew, 2003). Once again, this may be attributed to Bosentan being a mixed antagonist. At the 2007 10th international symposium on endothelin (ET-10) in Bergamo, Italy, several investigators pointed out that while mixed antagonists have had some effects in pre-clinical studies, overall these agents have had little to no effect in the clinical setting. Therefore, it was proposed that more specific ETrA antagonists may be more useful.

The first report on a new drug, produced by Actelion Pharmaceuticals, INC in Switzerland, Ro 61-1790 [5-methyl-pyridine-2-sulfonic acid 6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2-(2-1H-tetrazol-5-yl-+ ++pyridin-4-yl)-pyrimidin-4-ylamide] was published in 1997 (Roux et al., 1997). It was found to be 1000-fold more selective for ETrA than ETrB. It was suggested that Ro 61-1790, which was renamed Clazosentan, may be useful for TBI (Sato and Noble, 1998), ischemia (Dawson et al., 1999), and subarachnoid hemorrhage (Gorlach et al., 2001). In 2006, clazosentan was included in a clinical trial to prevent vasospasm following hemorrhage (Uhlmann, 2006). Interestingly, this drug has been shown to have little effect in non-brain areas (Vuurmans et al., 2004). Further, as shown in ***Figure 1***, we have compared the effects of BQ-123 (a selective ETrA antagonist) with both Bosentan and Clazosentan on their ability to improve CBF following TBI (all drugs given at 30 min post TBI) and found that Clazosentan recapitulates the data seen by BQ-123 administration while Bosentan did not improve CBF. Therefore, Clazosentan provides a great potential for controlling hypoperfusion following TBI.

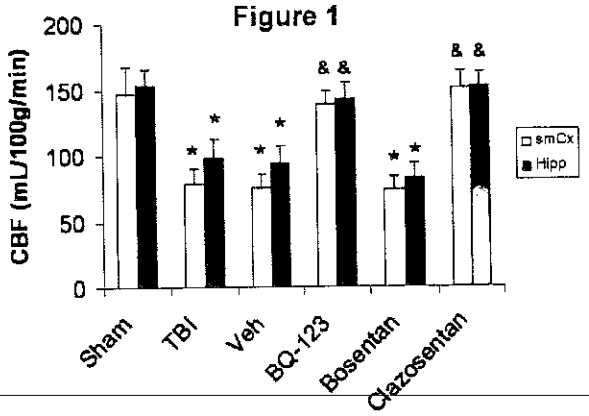


Figure 1. The effects of ETr antagonists on CBF following TBI.

Established information on safety, toxicity, absorption, and metabolism:

Clazosentan is currently undergoing Phase III clinical trial for use after aneurysmal subarachnoid hemorrhage. Therefore, much of the pharmacokinetic and safety information has been already published. Further, due to the relationship between Dr. Kreipke and Actelion, other pertinent information will be included in the application for IND. The following is a brief summary of findings:

Early investigations (summarized in Vatter et al., 2005a,b) showed that, unique to all endothelin class drugs, Clazosentan had a strong affinity to ETrA (2000X more efficacious to ETrA than ETrB), which makes this a potentially superior drug over other "mixed" antagonists which block both vasoconstrictory and vasodilatory properties of brain vascularization. In an initial investigation of Clazosentan in humans, drug was infused at doses of 3-60 mg/h for 3h, 60 mg/h for 6 h and at 30 mg/h for 12h. Each dose was

given to a separate group of subjects, six of whom received clazosentan and two placebo. Vital signs, ECG, adverse events, and clinical laboratory variables were monitored to assess tolerability. Blood and urine samples were collected frequently for pharmacokinetic and pharmacodynamic determinations. Infusion of clazosentan up to doses of 30 mg/h for 3h was well tolerated. A dose of 60 mg/h and longer infusions were less well tolerated and three subjects did not complete the 12h infusion of 30 mg/h due to adverse events. Headache was the most commonly reported adverse event followed by nausea, vomiting, and nasal congestion. The pharmacokinetics of clazosentan was dose proportional in the dose range investigated. Values (mean and 95% confidence intervals) for clearance and volume of distribution at a dose of 10 mg/h for 3h were 42.2 (36.6, 48.7) l/h and 32.4 (27.0, 38.8) l, respectively. Both variables were independent of dose. The elimination of clazosentan was characterized by a very rapid disposition phase with a half-life of 6-10min. Compared to baseline, endothelin-1 concentrations increased approximately 2-fold after infusion of clazosentan but no dose-dependent relationship could be discerned for this effect (van Giersbergen et al., 2007a).

Distribution, metabolism and excretion of clazosentan were investigated in four healthy male subjects given IV administration of 1 mg/kg ^{14}C-labeled drug. Blood, urine and feces samples were collected for a period of 8 days. Clazosentan was mainly excreted unchanged into feces whereas about 15% of the radioactive dose was recovered in urine. No metabolites representing more than 5% of total radioactivity were identified. No relevant inhibition of the human cytochrome P450 isoenzymes, 1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4, was observed in vitro at clazosentan concentrations largely exceeding those observed in clinical trials. In human blood, clazosentan was highly bound to plasma proteins and showed no significant penetration into red blood cells. Taken together, these results suggest that Clazosentan is a promising drug for use in clinical trials (van Giersbergen et al., 2009).

In 2005, 34 patients (61% female) with SAH were enrolled to study safety of Clazosentan. 16 received Clazosentan and 18 received placebo control. Overall, the rates, nature, and severity of adverse events were comparable between the two treatment groups. No adverse event pattern indicated a specific organ toxicity of clazosentan. Further the incidence of arterial hypotension was NOT greater than placebo (Vajkoczy et al., 2005). In 2007, these findings were extended to include a separate ethnic comparison (Japanese v. Caucasian). In this study which included 12 Japanese and 12 Caucasian males and females aged 18-50, Headache was the most frequent adverse event, and its incidence was similar in both ethnic groups. Both groups also reported dizziness and feeling hot with similar frequency. In addition to these 3 adverse events, Caucasian subjects incidentally reported other adverse events whereas no other adverse events were reported by Japanese subjects. Three of the 42 reported adverse events were of moderate intensity (all in Caucasian subjects), whereas all others were rated as mild by the investigator. The type of adverse events reported as similar between male and female subjects within each ethnic group, but females reported more adverse events than males (25 vs 17, respectively). Most subjects reporting headache were administered acetaminophen for pain relief. In addition to receiving acetaminophen to treat a headache, 1 Caucasian male subject was administered a single 10-mg dose of metoclopramide to treat nausea. All adverse events resolved without sequelae (van Giersbergen et al., 2007b).

C. Preliminary Data

In Specific AIM 1 we will determine a dose of Clazosentan that blocks the observed hypoperfusion following TBI. Figure 1 shows preliminary data in which 1 mg/kg Clazosentan was administered IV at 2 h post injury. This dose appears to be effective in blocking the hypoperfusion.

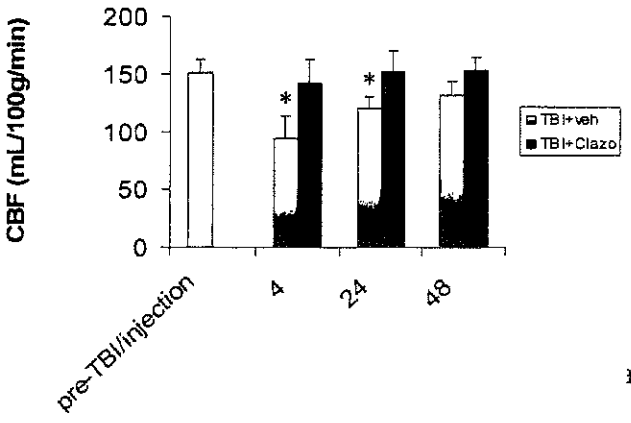


Figure 2. CBF was measured in 8 rats prior to injury (pre-TBI/injection, white) using ASL-MRI. Either veh (black and white) or 1 mg/kg Clazosentan (blue) was given IV at 2 h post TBI. Preliminary results (N=4 per group) show that Clazosentan is able to ameliorate TBI-induced hypoperfusion. *Similar results were detected in sensorimotor cortex (data not shown for space consideration).* *p<0.05 as compared with pre-TBI (analyzed using ANOVA with LSD post hoc).

We will also aim to determine a dose which is effective in improving behavioral deficits following TBI. Therefore we also tested performance on a radial arm maze following an injection of 1 mg/kg Clazosentan at 2 h post injury. While conducted in only a limited number of animals (N=6 for Clazo, N=8 for Sham and N =10 for TBI), results suggest that 1 mg/kg Clazosentan leads to a partial improvement in behavioral outcome (i.e., rats did not perform as poorly as TBI animals, however were not as successful as sham operated animals). Therefore, further dosing and dosing regime will be required to optimize outcome.

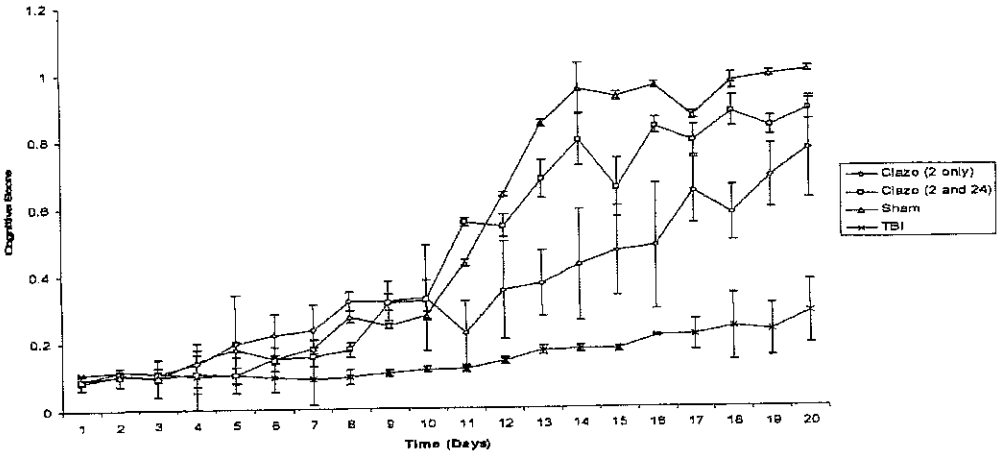


Figure 3. *Comparison of Clazosentan given at 2 hrs post TBI only versus at 2 and 24 hrs post TBI in rat. 4 groups of animals were used: sham operated controls (N=12), trauma only (N=13) (TBI), TBI + one injection of Clazosentan given at 2 hrs post TBI (Clazo (2 only)) (N=10), and TBI + two injections of Clazosentan given at 2 and 24 hrs post TBI (Clazo (2 and 24) (N=8). Data suggests that while Clazo (2 only) provides a partial improvement in behavioral outcome, Clazo (2 and 24) improves behavior to near sham control levels. All behavioral data in rats are expressed as the average over two trials per animal of an overall cognition score (cs) that incorporates latency (time it takes to retrieve all four Fruitloops™), spatial learning errors (error in which animal goes down an unbaited arm), or memory retention errors (error in which animal reenters a baited arm after food is already removed). Note that, while we are able to analyze each individual measure, we are now presenting data as cs to provide more consistent comparisons between rat and pig data. The cs is determined by the following equation: cs=1/{(0.77L)X[(0.063E1)+(0.031E2)+1]}, where L=latency in min, E1=spatial learning error, and E2=memory retention error. This equation was developed to incorporate both errors and latency into an overall assessment for cognition. This equation is based on trials with normal animals (no treatment) in which the fastest time on the maze in 1 min 18 seconds with no errors. These measures were designed, in part, to allow for an overall measure of performance and comparison of data across behavioral paradigms, across species.*

In Specific AIM 2 we want to recapitulate results gained from rat in a porcine model of TBI. In order to show that the porcine model mimics the rat model with regards to changes in CBF after TBI, we have included Figure 4 which shows that CBF, as in rats, is significantly reduced in pigs after TBI.

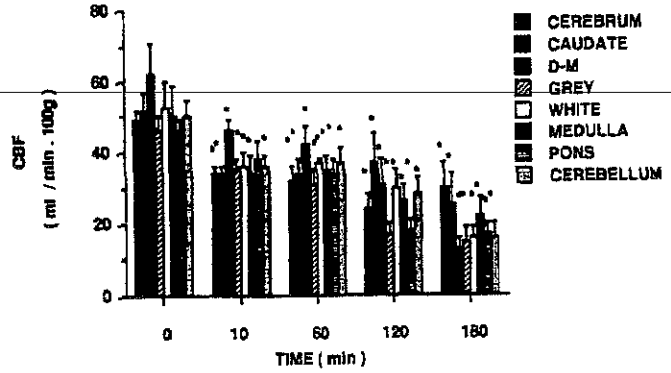


Figure 4. Influence of FPI (2 atm) on regional CBF in pigs, n=5. *p<0.05 compared to corresponding 0 time point (pre-injury) in multiple areas of the brain. Please note that, as in the rat, while we have the ability to measure multiple brain areas, in this proposal we will focus on hipp and smCx due to their known functional association with memory and learning.

Further, in order to show proof of concept that ETrA antagonism improves blood flow after TBI, Figure 5 shows the results of injection of BQ-123 in pigs at various time points following TBI. Results indicate that ETrA antagonism improves CBF following TBI in pigs.

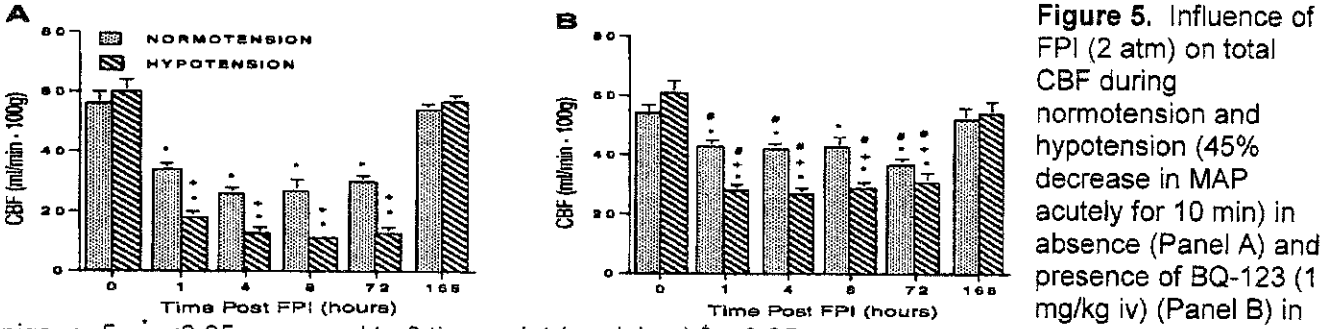


Figure 5. Influence of FPI (2 atm) on total CBF during normotension and hypotension (45% decrease in MAP acutely for 10 min) in absence (Panel A) and presence of BQ-123 (1 mg/kg iv) (Panel B) in pigs, n=5. *p<0.05 compared to 0 time point (pre-injury) #p<0.05 compared to corresponding normotension value #p<0.05 compared to corresponding non BQ-123 treated value (Panel A).

Neurofunctional assessments (balance beam, open field and T-maze) will be performed one day prior to injury and on days 3 and 7 after injury to evaluate motor skills, memory, learning, visual discrimination and "executive function" (Freiss et al., 2007) . We will use the open field and T-maze data to evaluate a Composite Cognitive Dysfunction (CCD) score (Freiss et al., 2009) which combines measures of behavior, executive function, memory, learning, reverse learning, and problem solving, and also correlates with severity of neuropathology, such that CCD≤3 is defined as impaired. Based on exciting new preliminary studies (see text below) demonstrating significant differences between shams and TBI piglets (N=5-7/group), ***we now supplement our published methods and analyses for open field with a novel analysis of movement persistence****. Using 9 zones, we evaluated open field position every 2 sec for TBI and sham animals, and characterized the probability that if the piglet is in a zone that it will remain in that zone at the next time point, (Pdiag). A Pdiag value of 1 indicates a stable position. For planned group sizes of 30 in Aim 2, our new pilot data obtained 3 and 7 days post-TBI (Pdiag 0.61±.05 and 0.53±0.06, respectively) indicates that injured piglets behavior is significantly more deliberate and less exploratory than shams or pre-injury assessments (0.44-0.46±.03). Similarly, cognitively impaired individuals are inactive for longer periods and interact with and explore objects less (Head et al., 1997).*

We ***have also modified our published T-maze methods to assess visual discrimination****. Briefly, we placed an image of three large black dots on the wall of the arm with the food reward, and an image of a single large black dot on the non-reward arm. On the last day of testing, piglets are allowed one minute to explore a walled 4'x8' test space with the images on opposing walls, and nose nudges on each image are counted. Our new preliminary data reveal that sham and injured piglets nudge the one-dot image with equal frequency (1±.38, 1±.6, respectively), but shams nudge the 3-dot image more than injured (2.9±.94, 1±.46, respectively) and more than the one-dot image, suggesting that injured animals have difficulty visually discriminating between the images and recalling the association between image and food reward.*

Finally, ***we have also added a 20° 9-inch wide inclined beam test to assess motor skills.*** *With food as a reward at the end of a 4' beam, piglets have five 20-sec trials, and a gait score is assigned for the test day if the piglet walks at least halfway to the reward on at least 2 trials. The 4-point gait scale ranges from 0 (2 or more slips on every beam trial) to 4 (no slipping or other walking difficulties on any trials). Injured piglets (1.1±.4 and 1.6±.5 on post-TBI day1 and 4) had lower gait scores than shams (3.4±.2 and 2.5±.2 on day 1 and 4) and pre-injury tests (2.5±.5), consistent with motor deficits in the injured animals. With an expected group size of 30* ***each of these new neurofunctional measures has adequate detectable effect sizes to distinguish between injured and sham animals****.*

D. Approach: Research Design and Methods

SPECIFIC AIM 1. Test whether a certain dose of Clazosentan, given as either single or multiple bolus injections at a range of timepoints post TBI will be effective in reducing the extent and duration of hypoperfusion and improve behavioral outcome in a rodent model of TBI.

Goals and timeline for *YEAR 1*: Determine whether candidate pharmacotherapy has characteristics which warrant proceeding to testing in a porcine model. ***(10months)***

Rationale for *Year 1*: Published and preliminary data from our laboratories show that endothelin-1, a powerful vasoconstrictor, is upregulated following TBI. Further, we have shown that one of its receptors, ETrA, which mediates vasoconstriction, is upregulated as early as 1 h post injury. This finding temporally corresponds with observed vasoconstriction and hypoperfusion. Data from several laboratories shows

that BQ-123, a non-clinically relevant ETrA antagonist, blocks TBI induced vasoconstriction. A recent publication from Dr. Kreipke's laboratory shows that blockade of ETrA using BQ-123, ameliorates hypoperfusion and reduces the extent of cell injury (Kreipke et al., 2009). Furthermore, preliminary data shows that BQ-123 improves behavioral outcome (Kreipke et al., 2009). With the recent production of a clinically relevant ETrA antagonist, and with the mutual agreement between Actelion and Dr. Kreipke to use this drug, we have generated preliminary data for this U01 application which shows that an IV injection of Clazosentan given 2 h following TBI recapitulates the results of BQ-123. Therefore, if funded, we will extend these findings to determine the optimal dose of Clazosentan needed *and the optimal range of times (2, 4, 8 or 12 h post injury)* that can be given to improve outcome.

Experimental Approach for *Year 1*:

Single bolus, 2, 4, 8, or 12 h for CBF and behavior:

In order to establish baseline, 24 h prior to TBI, all rats (360 animals) will have an initial ASL scan to determine CBF (CBF measurements will be taken as described in General Methods) in sensorimotor cortex (smCx) and hippocampus (hipp) due to the association of these anatomical areas with learning and memory. Animals (15 per group, groups = sham, TBI only, TBI+veh, TBI+0.1mg/kg, TBI+1.0mg/kg, TBI+10mg/kg Clazosentan) will be either sham operated or given TBI using the weight drop method described in General Methods. Following surgery, animals will be allowed to recover for 2, 4, 8, or 12 hours at which time they will receive no injection (TBI only), veh, or one IV injection of the selected dose of Clazosentan. Dosing range is based on established protocols in animals (Vater et al., 2005a,b) and on tolerability and safety data in humans involved in the SAH clinical trial (Vajkoczy et al., 2005; van Giersbergen et al., 2007a,b; 2009). At 4 h post drug injection a subset of 6 animals per group will be randomly selected for ASL CBF measurements. Animals will then be returned to home facility. At 24 h post injury, CBF measurements will be retaken in the same subset of 6 animals per group. Four h post CBF measurement (to allow for full recovery from anesthesia) all animals (15 per group) will be tested for motoric performance. As described below, any animal not performing at normal, non-treated levels will be discarded as this will confound interpretation of behavioral data. At 48 h post injury, CBF will be measured once again in the same subset of 6 animals per group as before. Four h after CBF measurements, behavioral data acquisition will commence. All animals (15 per group) will be tested for cognitive performance using the radial arm maze as described below in General Methods for 20 days (testing done from day 2 through day 20). Comparison across groups will be conducted using ANOVA with LSD post hoc to determine whether Clazosentan has an effect on either CBF or cognitive behavior following TBI.

-24h	0h	2, 4, 8 or 12 h	4h post injection	24h	28h	48h	52h	22d

ASL CBF → TBI/sham → injection → ASL CBF → ASL CBF → motoric testing → ASL CBF → behavioral testing → end

Single bolus, 2, 4, 8, or 12 h for Histology:

An additional six animals per group will be used to determine the extent of neuroprotection of Clazosentan. Animals will be treated as above, however no scans will be taken. At 48 h post injury animals will be perfused and brain tissue collected for histological analysis using H&E, acid fuschin, and fluoroJade, all standard histological preparations, to determine the extent of cell injury. After behavior has ceased, all animals will be perfused for histological analysis at a later timepoint (22 days after TBI) to confirm the extent of neuroprotection.

Two injection approach, 2, 4, 8, or 12 h + 24 h:

Same as single bolus approach, however an additional IV injection of Clazosentan will be given at 24 h. Second injection will match the dose of the first injection (e.g., 1.0 mg/kg given at 2 h and 24 h).

-24h	0h	2, 4, 8, 12h	4h Post injection	24h	25h	28h	48h	52h	22d

ASL CBF→ TBI/sham → 1st injection → ASL CBF → 2nd injection →ASL CBF → motoric testing → ASL CBF → behavioral testing→ end

Milestone for *Year 1*: A particular dose and dosing regime of Clazosentan will reduce the extent and duration of hypoperfusion and will improve behavioral outcome after TBI. *If* determined, *then* we will proceed to Specific Aim 2, Year 2.

Criteria for success for *Year 1*: TBI results in an approximately 30-35% reduction in blood flow followed by vasospasm in rats. This observation is recapitulated in humans. Therefore, we will quantify CBF using ASL-MRI, a technique routinely used in both small animal models as well as humans, following drug administration. Improvement will be defined as an increase in CBF of 20% or greater, thus reducing

hypoperfusion to 15-20% following TBI. Success will be defined by 70% of the animals per treatment group achieving this improvement. Furthermore, published data from our laboratory shows that TBI extends the time needed to learn behavioral tasks by minimally 7 days and increases number of errors by 20%, which when combined to produce a cognitive score (cs) (see statistical methods for derivation of equation) results in a reduction in cs by >40% (or <60% of control). Therefore, in the same animals in which CBF is determined, we will test behavioral outcome following drug treatment. Improvement in behavioral testing will be defined as an increase in cs to >60% of control. Success will be defined by 70% of the animals per treatment group reaching this improvement goal. *Improvement based on histological analysis will be defined as a reduction in acidophilic neuclei or FluoroJade-positive cell bodies (see General Methods) by 20%. Success will be defined by 70% of the animals per treatment group reaching this improvement goal.*

SPECIFIC AIM 2. Test whether a certain dose of Clazosentan, given as either single or multiple bolus injections at either *an early or later timepoint (to be determined by Year 1 rat studies)* post TBI will be effective in reducing the extent and duration of hypoperfusion and improve behavioral outcome in a porcine model of TBI.

Goals and timeline for *YEAR 2*: Determine whether candidate pharmacotherapy given at an early timepoint post injury has characteristics (i.e., improves CBF, *histopathological* and behavioral outcome) that would warrant testing a later injection time. ***(12 months)***

Rationale for *YEAR 2*: Many TBI studies use rodent models. However, rodents have a paucity of white matter. Pigs provide many advantages in modeling the human brain. The overall shape, gyral pattern, and distribution of gray and white matter are similar in pigs and humans. The growth pattern of the postnatal brain is similar to that of human infants. The response of the pig to hypoxia and ischemia parallels that observed in humans. CBF, metabolism, and maturation of pigs is similar to the human. Selective white matter vulnerability in humans similarly occurs in pigs with acute subdural hematoma. The gyrencephalic pig brain containing substantial white matter is appropriate to model human TBI. Due to these potential advantages in choosing a porcine model, we will aim to recapitulate the data gained in Year 1 in rats. Therefore, we will measure CBF at the same time points, in the same regions, using the same technique (ASL-MRI) as outlined in the studies in rats in order to test the efficacy of Clazosentan given at an early timepoint post injury in two distinct animal models of diffuse brain injury. Further, we will test behavior in the same animals, using a similar apparatus as that used in rat but designed for the pig. *Histology will be conducted identically to that in rat.*

Experimental Approach for *Year 2*:

If, as our preliminary data suggests, we are able to determine an effective dose and "window of opportunity" for Clazosentan in the rat model of TBI, we will then extend these results to include data from a more human-like model, the pig. The dosing range that will be studied in the pig studies will be derived from the information that we determined in the rat. Thus, the rat studies will inform the design of the pig studies. In Table 1, for example, D1 will be the dose found to be effective in the rat, while D2 may be 3 X D1, in order to gain further dose-response information in the pig. In year 2, animals will receive fluid percussion brain injury and will be treated either at *an early timepoint or an early timepoint followed by injection 24 hours post injury* (i.e. single bolus or multi-injection), and two doses will be examined (D1, D2) along with vehicle treated animals (Table 1, below). Studies will also be undertaken on sham operated animal receiving the same doses. Cerebral blood flow will be measured with ASL MRI the day prior to fluid percussion brain injury, 2 h following the first treatment as well as 48 h after injury (Table 1, below). Behavior will be evaluated at 72 h and 7 d. Histological analysis (as completed in rat studies) will be performed on all animals at the completion of behavior to test the neuroprotective properties of ETrA antagonism.

Group #	Year	Treatmt	Injury	Time Rx1	Time Rx2	Time Rx3	CBF	Behav	Histol
1	2	D1	Y	2	--	--	-1D, 6H, 48H	72H,7D	7D
2	2	D1	Y	2	8	--	-1D, 6H, 48H	72H,7D	7D
3	2	D1	Y	2	8	24	-1D, 6H, 48H	72H,7D	7D
4	2	D2	Y	2	--	--	-1D, 6H, 48H	72H,7D	7D
5	2	D2	Y	2	8	--	-1D, 6H, 48H	72H,7D	7D
6	2	D2	Y	2	8	24	-1D, 6H, 48H	72H,7D	7D
7	2	V	Y	2	8	24	-1D, 6H, 48H	72H,7D	7D
8	2	V	N	2	8	24	-1D, 6H, 48H	72H,7D	7D
9	3	D1	Y	--	8	--	-1D, 6H, 48H	72H,7D	7D
10	3	D1	Y	--	8	24	-1D, 6H, 48H	72H,7D	7D
11	3	D2	Y	--	8	--	-1D, 6H, 48H	72H,7D	7D
12	3	D2	Y	--	8	24	-1D, 6H, 48H	72H,7D	7D

D1 = dose 1; D2 = dose 2; V = vehicle; Y=yes; N=no; treatment times in hours; H=hours; D=days
Time Rx1, Rx2, Rx3 are time in hours following injury.

Milestone for *Year 2*: A particular dose and dosing regime of Clazosentan given at *an early timepoint* post TBI will reduce the extent of hypoperfusion and will improve behavioral outcome following injury. *If* determined, *then* we will proceed to injection of Clazosentan at *a later timepoint* post TBI in Year 3.

Criteria for success for *Year 2*: Using optimal dose and dosing regime as determined in rat in Year 1, improvement will be defined as a reduction in CBF to only 15-20% of baseline. Success will be defined by 70% of the pigs per group achieving the improvement goal. Behavioral deficits in pigs is defined as a composite cognitive dysfunction score (ccds). Poor outcome is defined as a ccds > 3. Therefore improvement will be defined by a reduction in the score below 3. Success will be defined by 50% of the animals per group improving below a 3 ccds when drug is administered at an early timepoint. Furthermore, a Pdiag <0.5 is indicative of impairment and, therefore, improvement will be defined as a reduction of the score to below 0.5. Success will be defined by 50% of the animals per group improving below a 0.5 Pdiag when drug is administered at an early timepoint. Overall success in behavior will be defined by meeting success criteria for each individual measure (e.g., success will be defined by improvement in both ccds and Pdiag). *Improvement based on histological analysis will be defined as a reduction in acidophilic neuclei or FluoroJade-positive cell bodies (see General Methods) by 20%. Success will be defined by 70% of the animals per treatment group reaching this improvement goal.*

Goals and timeline for *YEAR 3*: Determine whether candidate pharmacotherapy given at a later timepoint post injury has characteristics (i.e., improves CBF, *histopathological* and behavioral outcome) that would warrant a successful application for an IND ***(12 months)***

Rationale for *Year 3*: By testing administration of Clazosentan at a longer time point post TBI we will identify a diverse range of drug delivery times which will improve outcome.

Experimental Approach for *Year 3*:

In year 3, the first dose will be administered at *a later timepoint* than in Year 2 studies to examine the effect of delayed treatment with some groups treated also at 24 h (Table 1). In these animals CBF will be measured 2 h post first treatment and at 48 h. Behavior will be examined at the same times as outlined above for year 2 animals.

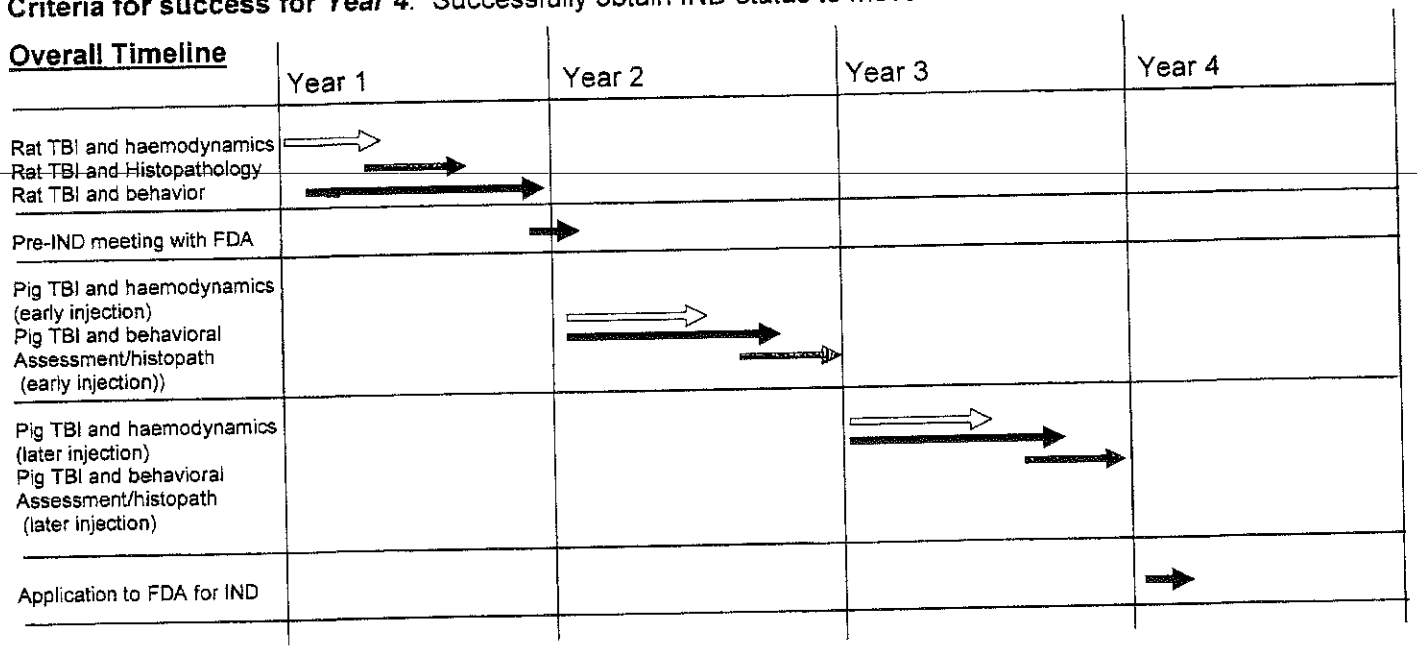
Milestone for *Year 3*: A particular dose and dosing regime of Clazosentan given at a later timepoint than that in Year 2 studies after TBI will reduce the extent of hypoperfusion and will improve behavioral outcome following injury. *If* determined, *then* we will proceed to Year 4 Terminal Milestone.

Criteria for success for *Year 3*: Using optimal dose and dosing regime as determined in rat in Year 1, success will be defined as in Year 2.

Terminal Milestone*: *Year 4 application for IND status with FDA

Goals and timeline for *Year 4*: Prepare application for IND ***(3 months)***

Criteria for success for *Year 4*: Successfully obtain IND status to move forward with clinical trial.



General Methods for YEAR 1 rat studies:

Closed head trauma model in rat:

Male Sprague-Dawley rats (Sprague-Dawley) will be anesthetized with 5% halothane in 2% oxygen prior to intubation, and then maintained on 1.5% halothane via a mask and spontaneous breathing. Halothane will be used as the anesthetic for all experiments. The use of halothane instead of the more recently introduced isofluorane is preferred because of recent evidence indicating the latter neuroprotective properties ([Zhao et al., 2007; Wei et al., 2007] and more recent data presented at the Brain '07, International Cerebral Blood Flow and Metabolism meeting held in Osaka, Japan). A midsagittal scalp incision will be performed and the underlying muscles retracted laterally. Cranioplastic cement will be used to attach a 10mm diameter X 3 mm thick, round metal helmet directly to the skull over the sagittal suture and between the coronal and lambdoidal sutures. The helmet is used to distribute the applied force over the surface of the parietal bones, thus preventing skull fractures with penetrating brain injury. After the cement is allowed to dry for three minutes, the animals will be placed prone on a platform as described in the acceleration impact trauma model of Marmarou (Marmarou et al., 1994). After 30-40 seconds of placement, 450g of weight contained in a hollow plastic cylinder will be dropped directly onto the helmet from a height of 2 meters. Following a brief convulsion and respiratory arrest, most animals restart breathing on their own. However, in some cases, the use of a rodent respirator or CPR is necessary prior to spontaneous breathing. Using these precautions, in our hands mortality has been reduced to less than 5%. In some animals after impact, the helmet will be removed and the skin sutured only if the skull shows no evidence of fractures. After suturing the skin, sensory cutaneous and evoked motor responses will be tested. Usually the intubation tube is removed at 10 minutes post trauma and only animals which are able to right themselves before 30 minutes after injury will be included in the study (Petrov et al., 2000; Petrov et al., 2002). Brain and leg muscle temperatures will be taken routinely, in some instances up to 24 hrs post injury. We have determined that brain temperature fluctuated only 1.5°C, and muscle temperature 1.3°C, during this time period.

CBF measurements:

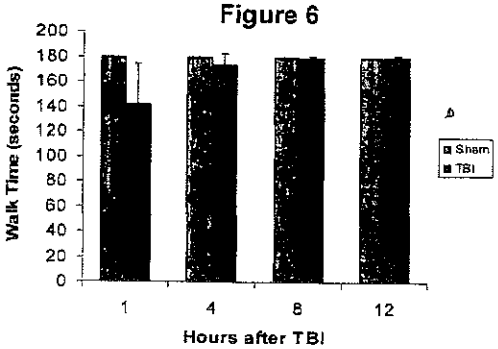
CBF measurements will be acquired using a standard protocol which is routinely used by our laboratory (Shen et al., 2007; Kreipke et al., 2009). Briefly, prior to image acquisition, anesthesia will be induced by a steady application of 1% halothane using a specially designed apparatus compatible with the MRI to sedate the animals. The animal will be placed in a prone position on a cradle with a custom-built palate holder equipped with an adjustable nose cone and stereotaxic ear bars in order to minimize movement during MRI scans.

The rat head will be positioned at the isocenter of a magnet. MRI scans will be repeated at four time points. Baseline scans will be run before TBI is induced, and then as indicated in the experimental design. All MRI measurements will be performed on a 4.7-T horizontal-bore magnetic resonance spectrometer (Bruker AVANCE) with an 11.6-cm-bore actively shielded gradient coil set capable of producing a magnetic field gradient of up to 250 mT/m. A whole-body birdcage radiofrequency (RF) coil (inner diameter, 72 mm) will be used as the transmitter for homogeneous RF excitation, and a surface coil (30 mm diameter) will be used as the receiver, with active RF decoupling to avoid signal interference. Two sequences will be run: T1 weighted image (used to identify brain regions and permit alignment of the two ASL-CBF scans that will be obtained in each animal) and ASL will be run for the measurement of flow. For all sequences, the field of view will be 40X40X24 mm^3; thus, the whole brain will be imaged. While we would, therefore, be able to measure any anatomical area in the brain, we will focus on smCx and Hipp due to their functional relationship to learning and memory. The remaining imaging parameters used are as follows: ASL: TR = 1550 ms, TE = 7.65 ms, matrix size NxXNy =128X70 (interpolated by zero filling in k-space to 256_256), slice=1 (thickness, 2 mm), Nacq=2, labeling slice=2 cm offset from isocenter, adiabatic fast passage with Magnetization transfer contrast (MTC) gradients=1.5 s, spin echo=3;

Assessment of motor deficit:

Since neurological deficits, while rarely seen using this model of TBI, would greatly hinder the ability of a rat to perform on the radial arm maze or in a Morris Water Maze, all animals will be screened following TBI for neurological outcome. In order to screen animals for motor deficit, all TBI animals will be tested using standard neurological function tests, including rotor rod performance, balance

Figure 6



beam, and ladder climbing. Based on preliminary screens (Figure 6), rats either performed well or, on the contrary, showed deficit on all tests and, therefore, animals performing at sub-control levels on any test will be grounds for removal from the study.

Behavioral testing and radial arm maze setup:

Behavioral testing will be conducted using a previously published protocol in our laboratory (Kreipke et al., 2008). Briefly, rats will be allowed to acclimate to their new environment (in DLAR facility) after their arrival. Then from day 1 to day 3 of the behavioral study the rats will be handled by the researcher for 10 to 15 minutes each. Acclimation to the maze environment also will be initiated during which the rats will be placed on the central platform of the radial arm maze and allowed to roam freely.

A custom designed radial arm maze will be built using black acrylic sheet (0.6 cm thick). Eight identical radial arms are fixed to an octagonal base platform that stands 63 cm above the floor. Each radial arm is 60 cm in length and 10 cm in width with 10 cm – high sidewalls along each arm. At the end of each arm a 5-cm end piece is placed. A hole measuring 2.5 cm in diameter is also cut 5 cm from the end of each radial arm to place a plastic food cup (1 oz). During behavioral testing, the maze is enclosed within four black linen walls. A white paper triangle (15-cm sides) is placed on one linen wall 10 cm above the base of radial arm #3. An 8" x 11" white paper square with bisecting black lines is placed on the same linen wall 10 cm above the base of radial arm #5. These visual cues are aimed to provide spatial guidance as to the location of the baited arm (i.e. containing the food).

The rats will be tested for the time taken to find the bait (half of a Fruit Loop cereal®) placed in a plastic cup of four different radial arms. Also the number of spatial learning (entering an unbaited arm) and memory retention (re-entering a baited arm after the food has been removed) errors will be recorded. All data is automatically obtained using an overhead camera linked to Smart™ Version 2.5 software (San Diego Instruments, San Diego, CA) Each rat will be tested daily for two consecutive time trials over a period of 20 days. The maximum time a rat will be allowed to spend in the maze is ten minutes per trial by the end of which is determined to be conclusion of a trial. Averages of these trials will be calculated and recorded.

Histology:

Cell injury will be assessed using standard histological preparations (H&E, Acid Fuschin, and FluoroJade (FJ) as previously described (Schmued et al., 1997)). For the former two methods, briefly 40 µm sections from smCx and hipp are rinsed in PBS, stained with either H&E or acid fuschin, dehydrated with increasing titers of EtOH (70-100%) and mounted with permount for analysis. For FJ, briefly 40 µm sections from smCx and hipp are washed in 80% ethanol with 1% NaOH, 70% ethanol and distilled water. Next tissue sections are washed in 0.06% $KmNO_4$ for 10 min and then washed in distilled water. FluoroJade solution (2 mL) is diluted in 48 mL 0.1% acetic acid and tissue is incubated for 30 min. Section are then rinsed in distilled water, dehydrated, air dried and mounted with a water based mounting media. Sections are analyzed using either a Nikkon light microscope (H&E and AF) or a Nikkon fluorescence microscope (FJ) with a Axiocam visualization package. Number of acidophillic nuclei or FJ-positive cell bodies are counted using Nikkon cell imaging software, averaged, and recorded to determine extent of cell injury.

General Methods for Years 2 and 3 porcine studies:

Closed head trauma model in pig.

Methods for brain FPI have been described previously (Armstead 1996). A device designed by the Medical College of Virginia is used. A small opening is made in the parietal skull. A metal shaft is sealed into the opening on top of intact dura. This shaft is connected to the transducer housing, which is in turn connected to the fluid percussion device. The device itself consists of an acrylic plastic cylindrical reservoir 60 cm long, 4.5 cm in diameter, and 0.5 cm thick. One end of the device is connected to the transducer housing, whereas the other end has an acrylic plastic piston mounted on O-rings. The exposed end of the piston is covered with a rubber pad. The entire system is filled with 0.9 % saline. The percussion device is supported by two brackets mounted on a platform. FPI is induced by striking the piston with a 4.8 kg pendulum. The intensity of the injury (1.9-2.1 atm. with a constant duration of 19-23 ms) is controlled by varying the height which the pendulum is allowed to fall. The pressure pulse of the injury is recorded on a storage oscilloscope triggered photoelectrically by the fall of the pendulum. The amplitude of the pressure pulse is used to determine the intensity of the injury.

Survival surgery of brain injured pigs.

Male pigs will be anesthetized with halothane (2-3 MAC) and aseptic technique used to surgically place the adaptor for connection to the brain injury device. After injury, the animal will be allowed to recover and

experimental protocols followed for behavioral assessment and determination of cerebral blood flow by ASL MRI. Six hours after FPI the pig will be anesthetized with halothane and taken to the MRI facility for measurement of blood flow (see descript of CBF Measurements below). After the imaging session, the animal will be returned to his cage for recovery, and kept warm (≈37° C) until awake. This procedure will be repeated 48 hours after FPI.

CBF measurements.

Cerebral blood flow will be measured using a pseudo-continuous arterial spin label (pCASL) technique routinely used in the Center for Functional Neuroimaging. For all three sessions (prior to FPI and 6 (or 8) and 48 h post FPI), the pig will be anesthetized with halothane and placed into the MRI scanner (Siemens TIM Trio) and a volume transmit/8-channel receive knee coil positioned on the head. The head will be held steady using a MR-compatible stereotaxic headholder. This positioning device will permit the head to be positioned similarly for both imaging sessions. The following acquisition parameters will be used: gradient-echo echoplanar imaging with TR/TE = 4000/17 ms, FOV= 25 cm, matrix = 64 x 64, slice thickness = 4 mm (yielding 4 mm isotropic resolution), flip angle = 90°, labeling time = 2 sec with a post-label delay of 1.2 sec, 120 ta/control pairs (16 min). Pior to the pCASL measurement, a structural MR image will be acquired with 1 mm isotropic resolution using a T1-weighted magnetization-prepared rapid acquisition gradient echo (MP-RAGE) sequence. This image will be used to identify brain regions and permit alignment of the two ASL-CBF scans that will be obtained in each animal.

Behavioral measurements.

Our previously published neurofunctional assessments (open field and T-maze) (Freiss et al, 2007) will be performed on 3 days and 7 days post-injury to evaluate memory, learning and executive function. In addition to all injured treatment groups, uninjured sham animals will receive all behavior testing. These shams are used as the basis for the composite cognitive dysfunction (CCD) z-score calculations, described below. The animals will be fasted for at least 6 hours before the first test begins. Testing order will be randomly assigned, and all tests will be on DVD for scoring by a blinded evaluator. All procedures involve operant conditioning techniques with food as a positive reward. Each animal will be placed in an open field (4 x 8 ft) with various objects placed in predetermined locations. The behaviors (e.g. nudging toys, walking, sleeping) will be scored as present/absent for every minute-long interval (Martin, P. and P. Bateson, 1986). The test provides a measure of exploratory interest which involves a high level of sensory processing (Kelly et al., 2001), and an intact prefrontal cortical-striatal-pallidal circuit and concomitant cerebellar function (Pierce, K. and E. Courchesne, 2001). Cognitively impaired individuals are inactive for longer periods and interact with and explore objects less (Head et al., 1997) The T-maze test will assess memory and cognition by requiring that they recall the location of a food reward (Bolhuis et al., 2004). This task is profoundly affected in cognitively impaired individuals (Head et al., 1995). After successful training, a novel object will be placed in the food reward arm of the T-maze, and time in contact with the novel object and latency to food reward will be recorded. We find increased latency to food reward by injured animals compared to instrumented sham animals. Finally, the food reward will be switched as a test of executive function and reversal learning (Adams et al., 2000). Time needed to complete the maze and number of times incorrect choices made will be scored.

Neurobehavioral outcome measures in pigs can exhibit a wide range of variability within each animal group. To improve the description of the overall neurobehavioral performance of pigs, we have developed a ccd score to evaluate overall neurofunction relative to the sham animals (Friess et al., 2009). The basis for the composite score is a set of neurobehavioral tests with the most consistent responses among previous SHAM groups (coefficient of variance ≤ 40%). Five neurobehavioral measures are included: T-maze training pass rate, T-maze intra-maze change time in contact with novel object, latency to food reward for T-maze normal trials and T-maze reversal trials, and sniffing the walls from open field testing. Together, these outcome measures assess executive function, memory, learning, reverse learning, and problem solving. First, for each of the five measurements, mean and standard deviation for SHAM are calculated for each behavior. Next, each injured animals' performance on the selected behaviors is compared to the SHAM mean and standard deviation. The injured animal's score is calculated for each behavior by taking the differences between the individual animal's performance and the SHAM mean. The difference is then divided by the standard deviation of the SHAM group to obtain a z-score for that pig for that assessment. Negative scores are used for individual performances that are below the SHAM mean for T-maze intra-maze change, T-maze normal trials, and T-maze reversal trials. Negative scores are also

used for individual performances that are above the SHAM mean for T-maze pass rate and sniffing the walls in open field testing. Scores for each of the five behavior measures for an individual subject are summed to calculate the composite CCD score for that animal. Additionally, we now supplement our published methods and analyses for open field with a novel analysis of movement persistence. Furthermore, we have added a visual discrimination task and a motor assessment. These new measures are outlined in the revised preliminary data section.

Histology. *Histological examination will be conducted in an identical fashion as that in rat (see above)*

Statistical Analyses for all studies

Note: all statistical analyses will be conducted in accordance with Wayne State University's Biomedical Statistical Core facilities guidelines.

CBF measurements. All data pertaining to CBF are expressed as the average of scans taken independently. CBF is expressed as mL/100g/min. Between group analyses are accomplished using one-way analysis of variance (ANOVA) with least significant difference (LSD) post-hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05. As previously reported (Shen et al., 2007), in rats we were able to detect significant changes in CBF between groups using 6 animals per group with 95% power at $\alpha = 0.05$. The cerebral blood flow data in the pig will be analyzed in a similar fashion to that in the rat. CBF will be obtained by pCASL prior to the fluid percussion injury, as well as acutely (6 or 8 hrs depending upon treatment time) and chronically (48 hrs) post injury. The ASL-MRI technique provides quantitative data of CBF with 4 mm resolution in the main structures of interest (smCx and Hipp) as well as in most of the remainder of the brain since the axial field of view will be 48 mm. This will permit an ANOVA analysis with blood flow in each region post injury compared to that prior to injury. With expected variability of the blood flow data, we expect to be able to detect significant changes in CBF with 10 animals/group with a power of 80% and α=0.05, similar to prior studies.

Behavioral Assessments. All behavioral data in rats are expressed as the average over two trials per animal of an overall cognition score (cs) that incorporates latency (time it takes to retrieve all four Fruitloops™), spatial learning errors (error in which animal goes down an unbaited arm), or memory retention errors (error in which animal reenters a baited arm after food is already removed). The cs is determined by the following equation: cs=1/{(0.77L)X[(0.063E1)+(0.031E2)+1]}, where L=latency in min, E1=spatial learning error, and E2=memory retention error. This equation was developed to incorporate both errors and latency into an overall assessment for cognition. This equation is based on trials with normal animals (no treatment) in which the fastest time on the maze in 1 min 18 seconds with no errors. These measures were designed, in part, to allow for an overall measure of performance and comparison of data across behavioral paradigms, across species. Between group analyses are accomplished using one-way analysis of variance (ANOVA) with least significant difference (LSD) post-hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05. In rats, due to the variability in behavior amongst individual animals, we have previously determined (Kreipke 2004, 2007) that we can distinguish significant differences between TBI and control animals using 12 animals per group. Antagonist studies will require 12-15 animals per group to show an improved performance with power of 90%. This allows us to distinguish a difference in cs of 10 with 90% power at $\alpha = 0.05$. Additional rats may be required due to failure to exercise, death, or motoric disability. In pigs, behavioral data are expressed as a composite cognitive dysfunction score (ccds) as described above in the behavioral measurements section. With success defined for each animal as ccds<3, we will determine the average success rate for each group. Success greater than 50% will be required for a dosing regime to be considered for translation to clinical trials. All calculations for ccds assume a one-sided Type I error level of±=0.05 with 80% power. The preliminary data presented for pigs with diffuse white matter damage demonstrated a ccds of approximately 15.9 ± 22.6 in injured animals on Day 7. Thus, an effect size of 0.65 times the standard deviation corresponds to a difference of 14.7 points. The corresponding within-group (two-sided) 95% confidence intervals for a mean will be ±0.62 times the standard deviation for n=30.

Histology. *All histological analyses are conducted using 4 to 6 sections per animal with 3 to 6 areas of analysis per section (see methodology). Data is expressed as an average of each area of analysis. In between group analysis is accomplished using one-way analysis of variance (ANOVA), with least significant difference post-hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05. Based on variability in these data from our previous studies (Kreipke et al., 2010) we can distinguish a difference in FJ + cell bodies with 95% power at an α level of 0.05 with 4-6 rats per group.*

V. VERTEBRATE ANIMALS

The NIH-mandated five points regarding vertebrate animals are addressed as following:

RATS

1. **Provide a detailed description of the proposed use of the animals for the work outlined in the Research Design and Methods section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.**

   For all experiments, Male Sprague-Dawley rats will be used. Please see proposal for break-down of animal number per experiment.

2. **Justify the use of animals, the choice of species, and the numbers to be used. If animals are in short supply, costly, or to be used in large numbers, provide an additional rationale for their selection and numbers.**

   The choice to use male Sprague-Dawley rats is based on previous work both in our lab and in the labs cited in the research design. Due to careful use of animals for multiple experiments, no more than 396 male rats (includes 10% exclusion) will be used in total (see experimental design for specific numbers per experiment). Further, rats will be used because of their low cost and because of the large body of information that is now known about their basic neuroanatomy, physiology, and behavior. Rats have an extremely high resistance to infection and are small in size which precludes using large amounts of expensive agents. In addition, the Sprague-Dawley strain has been shown to display pathological changes comparable to those encountered in clinical conditions.

3. **Provide information on the veterinary care of the animals involved.**

   Adherence to IACUC guidelines will be maintained in the experimental treatment and housing of the animals. Housing is provided in an IACUC approved facility in the same buildings as the laboratories (Dr. Kreipke's laboratory and the Department of Animal Laboratory Research Testing Facility). Training in proper care and handling of animals, as provided by the Wayne State University Department of Laboratory Animal Resources, has been successfully completed by the applicant.

4. **Describe the procedures for ensuring that discomfort, distress, pain, and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain, and injury.**

   After brain injury, some animals may experience persisting respiratory difficulties, and will be ventilated as necessary. If this ailment lasts longer than 60 min, such animals will be euthanized with sodium pentobarbital (120 mg/kg, IP injection) consistent with our previous work and with the Panel on Euthanasia of the American Veterinary Medical Association. It is possible that some degree of pain and distress will be present as a consequence of impact on the skull. However, animals are typically awake, but quiet and relatively inactive after trauma. By 1 hour they are usually active and are capable of eating and drinking on their own, although a drop of approximately 7% in body weight is expected. Analgesics will not be used immediately after injury because they (1) interfere with measurements of cerebrovascular function, (2) have neuroprotective effects and (3) in our experience with humans, there is very little or no need for analgesics right after a severe head injury. The effects of analgesics would compromise the results from the proposed experiments.

5. **Describe any method of euthanasia to be used and the reason(s) for its selection. State whether this method is consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia. If not, include a scientific justification for not following the recommendations.**

Upon termination of a given testing period, rats will be euthanized with a lethal dose of sodium pentobarbital (120 mg/kg IP as above) and death will be assured by bilateral pneumothorax and severing the aorta.

PIGS

1. **Provide a detailed description of the proposed use of the animals for the work outlined in the Research Design and Methods section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.**

For all experiments, Male pigs will be used. Please see Table 1 in the Research Plan for break-down of animal number per experiment.

2. **Justify the use of animals, the choice of species, and the numbers to be used. If animals are in short supply, costly, or to be used in large numbers, provide an additional rationale for their selection and numbers.**

The choice to use pigs is based on previous work in our lab. Due to careful use of animals for multiple experiments, no more than 120 pigs will be used in total (see Table 1 of Research Plan). Many TBI studies use rodent models. However, rodents have a paucity of white matter. Pigs provide many advantages in modeling the human brain. The overall shape, gyral pattern, and distribution of gray and white matter are similar in pigs and humans. The growth pattern of the postnatal brain is similar to that of humans. The response of the pig to hypoxia and ischemia parallels that observed in humans. CBF, metabolism, and maturation of pigs is similar to the human. Selective white matter vulnerability in humans similarly occurs in pigs with acute subdural hematoma. Therefore, the gyrencephalic pig brain containing substantial white matter is appropriate to model human TBI.

3. **Provide information on the veterinary care of the animals involved.**

Adherence to IACUC guidelines will be maintained in the experimental treatment and housing of the animals. Housing is provided in an IACUC approved facility. Training in proper care and handling of animals, as provided by the University of Pennsylvania Department of Laboratory Animal Resources, has been successfully completed by the applicant.

4. **Describe the procedures for ensuring that discomfort, distress, pain, and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain, and injury.**

After brain injury, some animals may experience persisting respiratory difficulties, and will be ventilated as necessary. If this ailment lasts longer than 60 min, such animals will be euthanized with sodium pentobarbital (120 mg/kg, IP injection). It is possible that some degree of pain and distress will be present as a consequence of impact on the skull. However, animals are typically awake, but quiet and relatively inactive after trauma. By 1 hour they are usually active and are capable of eating and drinking on their own. Analgesics will not be used immediately after injury because they (1) interfere with measurements of cerebrovascular function, (2) have neuroprotective effects and (3) in our

experience with humans, there is very little or no need for analgesics right after a severe head injury. The effects of analgesics would compromise the results from the proposed experiments.

5. **Describe any method of euthanasia to be used and the reason(s) for its selection. State whether this method is consistent with the recommendations of the American Veterinary Medical Association (AVMA) *Guidelines on Euthanasia*. *If not, include a scientific justification* for not following the recommendations.**

Upon termination of a given testing period, pigs will be euthanized with a lethal dose of sodium pentobarbital (120 mg/kg IP as above) and death will be assured by bilateral pneumothorax and severing the aorta.

Multiple-PI leadership Plan and Responsibilities:

This proposal draws on the expertise of both Drs. Kreipke and Armstead in order to gain the most appropriate data required for initiation of IND status with FDA. Both Drs. Kreipke and Armstead are experts in TBI research as it relates to endothelin-mediated hypoperfusion. Their individualized expertise lies in the animal models used to mimic head trauma. Dr. Kreipke is an expert in the rat acceleration-impact model while Dr. Armstead is an expert in the porcine lateral fluid percussion model. Incorporating both cross-model and cross-species data will enhance the application to FDA.

The first year of this application will be conducted in rat only. Therefore, most of the responsibilities will lie within Dr. Kreipke's team of experts which includes Dr. Dore-Duffy (an expert in hemodynamics). Dr. Kuhn (an expert in pharmacology), Dr. Rafols (an expert in the TBI model), Dr. Meuller (an expert in cardiovascular control) and Dr. Haacke (a leading authority in MRI imaging). However, Dr. Armstead will be included in Year 1 at 15% to allow for him to be involved in all data interpretation as the data gleaned from Year 1 will, in part, impact the direction of subsequent years. Dr. Armstead will have full access to data and we will meet on a biweekly basis by phone/video conference and twice throughout the year in person.

The second and third years of this application will be conducted in pig only. Therefore, most of the responsibilities will lie within Dr. Armstead's team of experts which includes Joel Greenberg (an expert in hemodynamics), Susan Margulies (an expert in pig behavior) and Douglas Smith (an expert in pig histopathology after TBI). In these years Dr. Kreipke will be retained at 15% to allow for involvement in interpreting and writing up the obtained results. He, too, will meet on a biweekly basis to discuss data and will meet twice in person to aide in data transfer and continuity.

Drs. Kreipke and Armstead have been in discussion for over two years regarding specifically this proposal. In this time, both have solidified their own respective teams. Further, negotiations with Actelion regarding material transfer and intellectual rights were accomplished (copy of MTA included in appendix). While Dr. Kreipke is directly implicated in the MTA, he will allow free and clear access to Clazosentan as needed specifically for this proposal. Drs. Kreipke and Armstead have also begun working together on projects related to endothelin, the results of which will be published in two manuscripts in an up-in-coming special edition of Neurological Research.

Conflict Resolution

While Drs. Kreipke and Armstead have an established working relationship and are both highly motivated to see the completion of all work and successful application to IND, both investigators have preemptively anticipated steps to resolve any conflict in interpretation of data that may arise. Drs. Harry Goshgarian from Wayne State University (an expert in spinal cord injury) and Andrew Kofke from University of Pennsylvania (an expert in human brain injury) will be available as needed as an independent set of investigators to aide in resolving any conflicts that may arise. If conflict arises, both multi-Pis will meet weekly with Drs. Goshgarian and Kofke to resolve and issues in a timely and scientifically-driven manner. Experience shows, however, that the joint collaboration between Drs. Kreipke and Armstead is likely to be highly successful.

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OMB No. 0925-0001

Form Approved Through 11/30/2010

Department of Health and Human Services
Public Health Services

Grant Application

Do not exceed character length restrictions indicated.

LEAVE BLANK—FOR PHS USE ONLY.		
Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT *(Do not exceed 81 characters, including spaces and punctuation.)*
Clazosentan: A novel treatment of traumatic brain injury

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☒ NO ☐ YES
(If "Yes," state number and title)
Number: Title:

3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR — New Investigator ☒ No ☐ Yes

3a. NAME (Last, first, middle): Armstead, William

3b. DEGREE(S): PhD

3h. eRA Commons User Name: ARMSTEADW

3c. POSITION TITLE: Research Professor

3d. MAILING ADDRESS *(Street, city, state, zip code)*:
Dept. of Anesthesiology and Critical Care
3620 Hamilton Walk; 339 John Morgan Bldg
Philadelphia, PA 19104-6112

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT: Anesthesia

3f. MAJOR SUBDIVISION: School of Medicine

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: 215-573-3674 FAX: 215-349-5078

E-MAIL ADDRESS: william.armstead@uphs.upenn.edu

4. HUMAN SUBJECTS RESEARCH ☒ No ☐ Yes
4a. Research Exempt ☐ No ☐ Yes — If "Yes," Exemption No.
4b. Federal-Wide Assurance No.
4c. Clinical Trial ☐ No ☐ Yes
4d. NIH-defined Phase III Clinical Trial ☐ No ☐ Yes

5. VERTEBRATE ANIMALS ☐ No ☒ Yes — 5a. Animal Welfare Assurance No. A309-01

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
07/01/2010	06/30/2015	$29,674	$47,478	$1,792,070	$2,822,311

9. APPLICANT ORGANIZATION
Name: Trustees of the University of Pennsylvania
Address: Office of Research Services
3451 Walnut Street, Rm. P-221
Philadelphia, PA 19104-6205

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☐ State ☐ Local
Private: → ☒ Private Nonprofit
For-profit: → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
1231352685A1
DUNS NO. 04-225-0712 Cong. District PA-002

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name: Pamela Caudill
Title: Executive Director
Address: Office of Research Services
3451 Walnut Street; Rm. P-221
Philadelphia, PA 19104-6205
Tel: 21-898-7293 FAX: 215-898-9708
E-Mail: pennaors@lists.upenn.edu

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: Marianne Achenbach
Title: Director, Research Services
Address: Office of Research Services
3451 Walnut Street; Rm. P-221
Philadelphia, PA 19104-6205
Tel: 215-898-7293 FAX: 215-898-9708
E-Mail: pennaors@lists.upenn.edu

14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 13. *(In ink. "Per" signature not acceptable.)* Marianne Achenbach — DATE 10/27/10

PHS 398 (Rev. 11/07) Face Page Form Page 1

Program Director/Principal Investigator (Last, First, Middle):

CHECKLIST

TYPE OF APPLICATION *(Check all that apply.)*

☐ NEW application. *(This application is being submitted to the PHS for the first time.)*

☒ RESUBMISSION of application number: U01NS072045-01A1
(This application replaces a prior unfunded version of a new, renewal, or revision application.)

☐ RENEWAL of grant number:
(This application is to extend a funded grant beyond its current project period.)

☐ REVISION to grant number:
(This application is for additional funds to supplement a currently funded grant.)

☐ CHANGE of program director/principal investigator.
Name of former program director/principal investigator:

☐ CHANGE of Grantee Institution. Name of former institution:

☐ FOREIGN application ☐ Domestic Grant with foreign involvement List Country(ies) Involved:

INVENTIONS AND PATENTS *(Renewal appl. only)* ☐ No ☐ Yes
If "Yes," ☐ Previously reported ☐ Not previously reported

1. PROGRAM INCOME ***(See instructions.)***
All applications must indicate whether program income is anticipated during the period(s) for which grant support is request. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)

2. ASSURANCES/CERTIFICATIONS ***(See instructions.)***
In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable. Descriptions of individual assurances/certifications are provided in Part III and listed in Part I, 4.1 under Item 14. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

3. FACILITIES AND ADMINSTRATIVE COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

☒ DHHS Agreement dated: 6/23/10 ☐ No Facilities And Administrative Costs Requested.

☐ DHHS Agreement being negotiated with Regional Office.

☐ No DHHS Agreement, but rate established with Date

CALCULATION* *(The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)*

a. Initial budget period:	Amount of base $	29,674	x Rate applied	60.00	% = F&A costs	$	17,804
b. 02 year	Amount of base $	893,312	x Rate applied	60.00	% = F&A costs	$	535,987
c. 03 year	Amount of base $	741,683	x Rate applied	60.00	% = F&A costs	$	445,010
d. 04 year	Amount of base $	52,401	x Rate applied	60.00	% = F&A costs	$	31,440
e. 05 year	Amount of base $		x Rate applied		% = F&A costs	$	
					TOTAL F&A Costs	$	1,030,241

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.)*:

4. DISCLOSURE PERMISSION STATEMENT: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)? ☒ Yes ☐ No

WAYNE STATE UNIVERSITY
SCHOOL OF MEDICINE

DEPARTMENT OF ANATOMY
AND CELL BIOLOGY

October 25, 2010

To the Review Committee:

This letter is written to enthusiastically support the efforts of Dr. Christian Kreipke in his pursuit of U01 funding for the proposal entitled, **"Clazosentan: a novel treatment for traumatic brain injury."** Dr. Kreipke is a junior faculty member in the Department of Anatomy and Cell Biology, yet has proved to be productive, organized and able to manage his time effectively. He has also proven to possess independence and strong leadership skills for a junior faculty member, in that he has established multiple collaborations across departments, and managed a laboratory of his own with three research assistants, three rotating medical students and two undergraduates. This, combined with his own vitality, allays any concerns that I may have as to whether Dr. Kreipke is suited to carry out the proposed work in addition to his funded projects.

As Chair of the department, I am committed to supporting the continued success of Dr. Kreipke, as concretely demonstrated by start-up funds from the department, matched by funds both from the School of Medicine Research Office and the Office of the Vice President for Research of Wayne State University.

Sincerely yours,

Linda D. Hazlett, Ph.D.
Distinguished Professor and Chair

LDH/slh

540 EAST CANFIELD AVENUE ◊ GORDON H. SCOTT HALL OF BASIC MEDICAL SCIENCES ◊ DETROIT, MI 48202 ◊ (313) 577-1061 ◊ FAX (313) 577-3125

School of Medicine

Daniel A. Walz, Ph.D.
Office of Research
School of Medicine
Scott Hall, Suite 1261
540 East Canfield
Detroit, MI 48201
Tel. (313) 577-9553
FAX (313) 577-9399

October 29, 2010

To the Review Panel:

After reading Dr. Kreipke's proposal for a U01 entitled, "Clazosentan: a novel treatment of traumatic brain injury" we are very excited about the prospect of this type of research being conducted at Wayne State University in conjunction with University of Pennsylvania. Dr. Kreipke is a very bright and energetic young individual who, in my assessment, is most capable of carrying out this work, especially in light of the extensive collaborations he has secured throughout multiple departments in the School of Medicine.

The Office of Research at Wayne State University's School of Medicine is committed to assisting Dr. Kreipke as needed in his pursuit of this award. Upon successful funding we will continue this support both in time and resources. Furthermore, upon granting of IND status, my office is committed to working in close association with our Clinical Departments to assist with the transition to implementation of Clazosentan into human trials. We have already identified key faculty and staff who have extensive experience in clinical trials who will assist Dr. Kreipke. We wish him all the very best in the review process.

Sincerely,

Daniel A. Walz, Ph.D.
Associate Dean for
Research and Graduate Programs

ACTELION

Dr. Christian Kreipke

Assistant Professor, Anatomy

and Cell Biology

Wayne State University, School

of Medicine, Detroit, USA

Allschwil, 20 January 2010

Dear Dr. Kreipke,

We are very interested in your proposal to test the efficacy of clazosentan in animals models of traumatic brain injury (TBI) and will continue to provide you with clazosentan under a material transfer agreement. Such a study is the logical continuation of your promising early findings obtained with peptidic endothelin receptor antagonists in the same models. Positive outcome with clazosentan in your models would encourage us to envisage such an indication. Furthermore, the existing preclinical package including DMPK, safety pharmacology and toxicology of clazosentan would be appropriate since this new indication is close to SAH for which clazosentan is already in phase III.

Yours sincerely,

Marc Iglarz

Pharmacology and Preclinical development

Actelion Pharmaceuticals LTD.

Actelion Pharmaceuticals Ltd
Gewerbestrasse 16 | CH-4123 Allschwil | Switzerland | phone +41 61 565 65 65 | fax +41 61 565 89 03 | marc.iglarz@actelion.com
|www.actelion.com
Address for visitors: Hegenheimermattweg 91

WAYNE STATE UNIVERSITY

Paula Dore-Duffy, PhD
Professor, Neurology
3126 Elliman Clinical Research
Office: (313) 577-0354
email: pdduffy@med.wayne.edu

October 19, 2010

Dr. Christian W. Kreipke
Department of Anatomy and Cell Biology
Wayne State University, School of Medicine
540 E. Canfield, Rm. 9312
Detroit, MI 48201

Dear Chris:

I am writing this letter to confirm my willingness to serve as a Co-Investigator on your NIH application entitled, "Clazosentan: A Novel therapy for TBI". Specifically, I will assist you with characterization of any hemodynamics in your experimental models.

Yours truly,

Paula Dore-Duffy, PhD

Penn Medicine
University of Pennsylvania School of Medicine

Department of Neurology
Joel H. Greenberg, Ph.D.
Research Professor

October 21, 2010

William Armstead, Ph.D.
Research Professor
Department of Anesthesiology and Critical Care
University of Pennsylvania
Philadelphia, PA 19104

Dear Bill –

I am excited about the opportunity to collaborate with you on your subcontract with Wayne State University "Clazosentan: A novel treatment of traumatic brain injury". Our collaboration in the past has been extremely fruitful and I am delighted to be involved in this project with you.

As you know, the Cerebrovascular Research Center has been involved in animal models of stroke and shock for over three decades with particular interest in cerebral blood flow measurements following insult. We have all the facilities and expertise to successfully carry out the blood flow measurements with magnetic resonance imaging as described in the proposal.

I look forward to undertaking these studies with you.

Sincerely,

Joel H. Greenberg, Ph.D.
Research Professor of Neurology

WAYNE STATE UNIVERSITY
SCHOOL OF MEDICINE

Donald M. Kuhn, PhD, Professor
Department of Psychiatry & Behavioral Neurosciences,
Center for Molecular Medicine and Genetics, and
John D. Dingell VA Medical Center

Correspondence:
John D. Dingell VA Medical Center
Research Service (11R)
4646 John R, Detroit, MI 48201
Office: (313) 576-4457
Laboratory: (313) 577-4466
email: donald.kuhn@wayne.edu

October 20, 2010

Dr. Christian W. Kreipke
Department of Anatomy and Cell Biology
Wayne State University, School of Medicine
540 E. Canfield, Rm. 9312
Detroit, MI 48201

Dear Chris:

I am writing this letter to confirm my continued willingness to serve as a co-investigator on your NIH U01 application entitled, "Clazosentan: a novel treatment for traumatic brain injury". I was encouraged once again by your summary statements and feel that you have answered the critiques and hence have a much stronger application.

As you know, my laboratory has considerable experience in determining effective doses in pharmacological studies and I will be happy to assist you in your studies in any way possible. Our lab is open to you and you we will be happy to provide you with any needed refresher instruction in carrying out the relevant techniques relating to histopathology and pharmacological determination. Of course, you are also free to use the instrumentation in our lab in coordination with our ongoing studies. I have enjoyed our discussions on your proposal and I am sure that you will uncover many interesting and novel findings. I am looking forward to working with you. Good luck with the review of your final revision of this grant.

Yours truly,

Donald M. Kuhn, PhD

October 27, 2010

William Armstead, Ph.D.
Research Professor
Department of Anesthesiology and Critical Care
University of Pennsylvania
Philadelphia, PA 19104

Dear Bill,

I am excited about the opportunity to collaborate with you on your subcontract with Wayne State University "Clazosentan: A novel treatment of traumatic brain injury".

As you know, my laboratory has been involved in pig models of traumatic brain injury, with particular emphasis on determining behavioral indices of outcome. We have all the facilities and expertise to successfully carry out the pig fluid percussion brain injury behavioral studies as described in the proposal.

I look forward to undertaking these studies with you.

Sincerely,

SS Margulies

Susan S Margulies, Ph.D
Professor of Bioengineering

WAYNE STATE UNIVERSITY

Department of Physiology
Wayne State University School of Medicine
540 E. Canfield, 5263 Scott Hall
Detroit, MI 48201
pmueller@med.wayne.edu
Phone: (313) 577-1559
Fax: (313) 577-5494
http://www.med.wayne.edu/physiology

October 22, 2010

Christian Kreipke, Ph.D.
Department of Anatomy and Cell Biology
Wayne State University School of Medicine
501 E. Canfield, 9312 Scott Hall
Detroit, MI 48201

I am writing in continued enthusiastic support of your NIH U01 grant proposal entitled "Clazosentan: a novel treatment for traumatic brain injury" I am excited to provide my knowledge and experience to this project should your novel drug have any effect on peripheral circulation. Since laboratories for our group of investigators interested in control of the circulation are located in Scott Hall, the environment is excellent for interactions and cooperative efforts. Your interest and preliminary data on effects of the endothelin antagonist are highly novel and add a new dimension to research efforts in the area of traumatic brain injury.

I wish you every success with this interesting and important work. I wish you the best of luck with your final revision of this application.

Sincerely,

Patrick J. Mueller

Patrick J. Mueller, Ph.D.
Assistant Professor of Physiology

WAYNE STATE UNIVERSITY

Jose Rafols, PhD
Professor, Anatomy and Cell Biology
Wayne State University
Room 9320, Scott Hall
540 East Canfield
Detroit, MI 48201
jrafols@med.wayne.edu
313.993.4393 (o)

October 18, 2010

Dr. Christian Kreipke
Assistant Professor, Anatomy and Cell Biology
Wayne State University, School of Medicine

Dear Chris:

It is with great pleasure that I offer to you my expertise in assessing TBI-related histopathology as it pertains to your present U01 proposal entitled, "Clazosentan: a novel treatment for traumatic brain injury". It has been such a pleasure working with you on designing the required histology component of this revision. I will continue to offer both my expertise and physical capabilities in conducting H&E, VAF, FJ, etc. analyses.

On a personal note, I wish to state how proud I am of your accomplishments thus far. I feel that this U01 represents a culmination of both of our work. I am thrilled at the independence that you have shown since being my postdoctoral fellow. This being said, while I respect your independence, please know that you can still rely on me for any help in interpretation of your results.

I wish you all the best in this final revision of your U01 project.

Sincerely,

Jose A. Rafols, PhD

October 27, 2010

William Armstead, Ph.D.
Research Professor
Department of Anesthesiology and Critical Care
University of Pennsylvania
Philadelphia, PA 19104

Dear Bill,

I am excited about the opportunity to collaborate with you on your subcontract with Wayne State University "Clazosentan: A novel treatment of traumatic brain injury". Our collaboration in the past has been extremely fruitful and I am delighted to be involved in this project with you.

As you know, my laboratory has been involved in pig models of traumatic brain injury, with particular emphasis on determining histopathologic indices of outcome. We have all the facilities and expertise to successfully carry out the pig fluid percussion brain injury histopathology as described in the proposal.

I look forward to undertaking these studies with you.

Sincerely,

DH Smith

Douglas H. Smith, MD
Robert A. Groff Professor of Neurosurgery

WAYNE STATE UNIVERSITY

Harry Goshgarian, PhD
Professor
Anatomy and Cell Biology
9304 Scott Hall
540 East Canfield
Detroit, MI 48201
hgoshgarian@med.wayne.edu
(o) 313.577.1045
(f) 313.577.3125

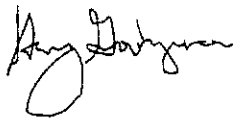
October 20, 2010

Christian Kreipke, PhD
Assistant Professor
Anatomy and Cell Biology

Dear Christian:

I have thoroughly enjoyed reading your revised application, "Clazosentan: A novel treatment of traumatic brain injury". I, therefore, would be happy to continue to serve on your "conflict resolution" committee should any disparity occur between you and Dr. Armstead over the interpretation of your results. Furthermore, I would be happy to assist you in any capacity during the tenure of this project. If you have any concerns, please feel free to contact me.

Sincerely,



Harry Goshgarian, PhD

Penn Medicine
Hospital *of the* University *of* Pennsylvania

Department of Anesthesiology and Critical Care

Department of Neurosurgery

W. Andrew Kofke, MD, MBA, FCCM
Professor of Anesthesiology and Critical Care
Director, Neuroanesthesia
Co-Director, Neurocritical Care

October 6, 2010

William M Armstead, Ph.D
Department of Anesthesiology and Critical Care
University of Pennsylvania

RE: Clazosentan: A novel treatment of traumatic brain injury

Bill,

I have read the above grant proposal and am enthusiastic regarding your goal of testing whether clazosentan is effective in ameliorating hypoperfusion and, ultimately, improving behavioral outcome following TBI. Therefore, please consider me available to serve on your "conflict resolution" committee should any disparity occur between you and Dr. Kreipke over the interpretation of your results. Furthermore, I would be happy to assist you in any capacity during the tenure of this project.

Yours truly,

W Andrew Kofke MD MBA FCCM

3400 Spruce Street | Philadelphia, PA 19104-4283 | 215.349.8993 | Fax: 215.615.3898 | kofkea@uphs.upenn.edu

WAYNE STATE UNIVERSITY

WSU MR RESEARCH FACILITY

Professor E. Mark Haacke, Ph.D., Director

Harper University Hospital
MRI Center Concourse
3990 John R
Detroit, MI 48201

Phone: 313-745-1395
Fax: 313-745-9028
Email: NMRIMAGING@aol.com

October 21, 2010

Dr. Christian W. Kreipke
Department of Anatomy and Cell Biology
Wayne State University, School of Medicine
540 E. Canfield, Rm. 9312
Detroit, MI 48201

Dear Chris:

This letter confirms my support as a consultant for your NIH U01 application entitled, "Clazosentan: a novel treatment for traumatic brain injury"". Though I am highly confident in your abilities to assess CBF using ASL, please know that if any complications arise I will be happy to assist you in any way that I can. I also want to assure you that you will have full access to the WSU 4.7 Tesla animal research scanner in the MRI facility and to the MR technician involved in running the scanner. In addition, I am happy to assist in preparing manuscripts and grant applications that directly arise from this proposal.

Yours truly,

E. Mark Haacke, PhD

The Center for Advanced Magnetic Resonance Imaging and Spectroscopy in the Department of Radiology currently operates several MRI scanners fully dedicated for research protocols. The scanner that will be used in the proposed porcine studies is a 3.0 Siemens TIM Trio. Gradient coils are capable of imaging at 40mT/m with slew rates in excess of 200 T/m/s. This scanner includes standard capabilities for echoplanar imaging, arterial spin labeled perfusion imaging, diffusion imaging, angiography, spectroscopy, and spectroscopic imaging. BOLD fMRI sequences include automatic higher order shimming and both prospective and retrospective motion correction. Gradient performance allows 4 mm isotropic voxels at TR=2 sec and 3 mm isotropic voxels at TR=3 sec. The 3T system has several transmit/receive volume head coils and a multi-element receive only volume coil suitable for parallel acquisition schemes. Image data from these scanners can be ported directly to CD or a local workstation for back-up onto DVD for larger data sets.

Data analysis will take place in the Center for Functional Imaging (CfN) (www.cfn.upenn.edu) which is a Type 1 Center within the Departments of Radiology and Neurology that provides infrastructure support for functional neuroimaging at the University of Pennsylvania. The CfN is comprised of investigators and staff with a broad range of expertise in neuroimaging including regulatory affairs, MRI methods development, MRI physics and pulse programming, instrumentation, experimental design, computing, and image analysis procedures.

The CfN data analysis cluster houses a total of 12 public workstations connected to 40+ CPUs running 32-bit x 86 (Linux) gigabit networking cluster. There is 40 terabyte online disk strorage in 3 RAID arrays to provide online storage, with backup via a 484-slot LTO Ultrium-3 tape library for periodic full backups and nightly incremental backups. Software to run a broad range of data preprocessing and analysis procedures is available, including VoxBo (developed at Penn), Matlab, IDL, SPM, FSL, AFNI, AIR, Brain Voyager, SNAP, FreeSurfer and others. Two rooms have a 42-inch plasma screen for meetings and presentations. A public (PennNet) server, including web service, mailing lists, ftp download, source code management, calendaring, database hosting and mediawiki-based wikis also serves the CfN community.

Histological Core

Core histology equipment available for this project include cryostats, microtomes, Nikon E600 microscope with bright field and fluorescent capabilities, Spot digital camera, and tissue processing equipment.

Behavioral Determination

Behavior studies will be performed in the Neurosurgical Trauma Laboratory located in the medical school complex, 3 blocks from Dr. Margulies' office in Hayden Hall and Injury Biomechanics lab in the Towne Building. There is a 45ft x 75ft x 24ft pen of 12 interlocking industrial plastic pieces that allows for behavior tests such as balance beam, open field, food cover, glass frustration and T-maze. Assessments are recorded with a Philips DVDR 75, a dual voltage color video camera with 4mm lens, and a Panasonic 20 inch television.

Year 4 IND

Both Drs. Kreipke and Armstead will consult with Stephen Goldner, owner of Regulatory Affairs Associates (www.regaffairs.net) located in Southfield, MI, a company that specializes in consulting for such matters as IND application